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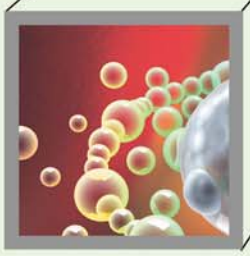
Enzymes

DNA Polymerase
Reverse Transcriptase
DNA Ligase
Restriction Enzymes



Enzymes
Phone: +82-42-930-8777 Email: reagents-support@bioneer.com

01 DNA Polymerase

DNA Polymerase

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Enzymes

Overview

Bioneer offers a wide range of enzymes for PCR, Reverse Transcription, Reverse Transcriptase PCR (RT-PCR) and DNA Ligation. Our Top DNA Polymerase is the fastest thermostable PCR polymerase on the market. We are the only company to apply a novel "enzyme-mediated" HotStart technology to our enzymes. In addition, our *CycleScript* Reverse Transcriptase uses a Cyclic-RT reaction method which procides robust cDNA synthesis.

For faster and easier reaction set up, *AccuPower*® PreMix Series contains lyophilized all required components for amplification in a sing tube.

Selection Guide

Choose the Bioneer Enzyme That's Right for You

	Application	DNA Polymerase			HotStart DNA Polymerase		*ProFi Taq* DNA Polymerase	Reverse transcriptase		
		TOP	*Taq*	*Pfu*	*TOP*	*Taq*		MMLV RT	CycleScript RTase	*RocketScript*™ RTase
PCR	Standard PCR	√	√		√	√	√		√	√
	HotStart PCR			√	√	√				
	High-fidelity PCR			√			√			
	Long Kb PCR			√			√			
	High sensivity				√					
	GC rich PCR				√	√				
	Multiplex PCR				√	√				
RT	Standard RT							√	√	√
	Cyclic RT								√	
	Standard RT/PCR									

Product Name	Product size	Yield	Specificity	Fidelity	GC-rich	Heat Stability	Leaves 3'-A
Top DNA Polymerase	~ 10 kb	*****	****	***	***	***	Yes
Taq DNA Polymerase	~ 10 kb	*****	****	***	***	***	Yes
HotStart DNA Polymerase	~12 kb	****	****	***	****	****	Yes
HotStart *Taq* DNA Polymerase	~12 kb	****	****	***	****	****	Yes
ProFi Taq DNA Polymerase	~30 Kb	****	****	***	****	****	Yes
Pfu DNA polymerase	~10 kb	**	***	****	**	***	No
M-MLV Reverse Transcriptase	~ 9 kb	***	-	-	***	**	-
CycleScript Reverse Transcriptase	~ 9 kb	****	-	-	***	***	-
RocketScript™ Reverse Transcriptase	~ 10 kb	****	-	-	***	****	-

01
DNA Polymerase

Top DNA Polymerase

■ Description

Top DNA polymerase is a novel thermostable DNA polymerase that is more processive than *Taq* DNA polymerase. In fact, the extension rate of *Top* DNA Polymerase is three times faster than that of Taq DNA Polymerase! *Top* DNA Polymerase can be used for a variety of PCR applications (including TA cloning) and is a robust enzyme for everyday PCR. There is not proofreading or 5'-3' Exonuclease activity.

■ Features and Benefits

- Fast:
  Three times processive than standard *Taq* DNA Polymerase
- High performance:
  Up to 10 kb amplification

■ Application

- Real-Time quantification of DNA and cDNA targets using SYBR Green dye.
- Gene expression profiling
- Microbial & viral pathogen detection

■ Enzyme Properties

- 5' to 3' exonuclease activity: No
- 3' to 5' exonuclease activity: No
- 3' – A overhang: Yes
- Amplification: Up to 10 kb

■ Reagents Supplied

10 x Reaction b uffer with (or without) $MgCl_2$: Tris (pH 9.0), 15 mM $MgCl_2$, etc
1 x Dilution buffer: 50% glycerol containing 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, pH 8.0
dNTP Mix: 2.5 mM of each dNTP

■ Concentration

500 U/100 µl

■ Storage Conditions

50% glycerol containing 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, pH 8.0

■ Storage Temperature

-20℃

■ Unit Definition

One unit is defined at the amount of enzyme that will incorporate 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72℃

■ Experimental Data

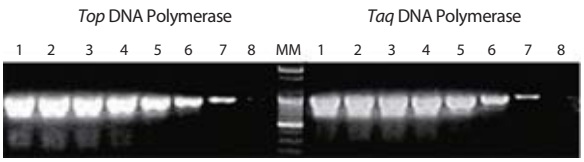

Figure 1. Sensitivity test of *Top* DNA polymerase and *Taq* DNA polymerase using Lambda genomic DNA.
Each fragment was amplified from a template dilution series (100 ng to 10 fg DNA per reaction) using 1 U of each DNA Polymerase.

Lane 1: 100 ng Lambda genomic DNA
Lane 2: 10 ng Lambda genomic DNA
Lane 3: 1 ng Lambda genomic DNA
Lane 4: 100 pg Lambda genomic DNA
Lane 5: 10 pg Lambda genomic DNA
Lane 6: 1 pg Lambda genomic DNA
Lane 7: 100 fg Lambda genomic DNA
Lane 8: 10 fg Lambda genomic DNA
Lane MW: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

Top DNA Polymerase

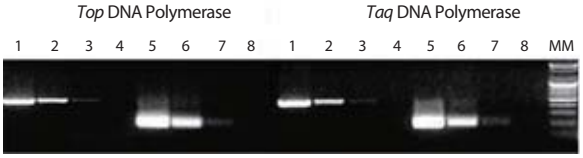

Figure 2. Sensitivity test of *Top* DNA Polymerase and *Taq* DNA polymerase using bacterial and human genomic DNA.
A 500 bp fragment was amplified from a bacterial genomic DNA dilution series (Lane 1-4: 1 ng to 1 pg per reaction) and a 220 bp fragment was amplified from a human genomic DNA dilution series (Lane 5-8: 10 ng to 10 pg per reaction). 1 U of each DNA Polymerase was used for all reactions.

Lane 1: 1 ng bacterial genomic DNA
Lane 2: 100 pg bacterial genomic DNA
Lane 3: 10 pg bacterial genomic DNA
Lane 4: 1 pg bacterial genomic DNA
Lane 5: 10 ng human genomic DNA
Lane 6: 1 ng human genomic DNA
Lane 7: 100 pg human genomic DNA
Lane 8: 10 pg human genomic DNA
Lane MW : 100 bp plus DNA Ladder (Bioneer, Cat. No. D-1035)

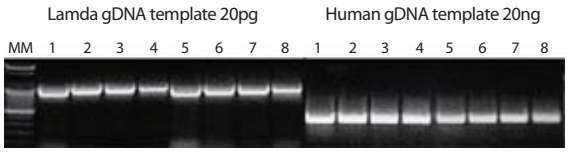

Figure 3. Enzyme activity test of *Top* DNA Polymerase and *Taq* DNA polymerase.
Top DNA polymerase/ *Taq* DNA polymerase was serially diluted and used to amplify 20 ng of each lambda and human genomic DNA.

Lane 1: 1 U of Top DNA Polymerase used
Lane 2: 0.5 U of Top DNA Polymerase used
Lane 3: 0.33 U of Top DNA Polymerase used
Lane 4: 0.25 U of Top DNA Polymerase used
Lane 5: 1 U of Taq DNA Polymerase used
Lane 6: 0.5 U of Taq DNA Polymerase used
Lane 7: 0.33 U of Taq DNA Polymerase used
Lane 8: 0.25 U of Taq DNA Polymerase used
Lane MW : 100 bp plus DNA Ladder (Bioneer, Cat. No. D-1035)

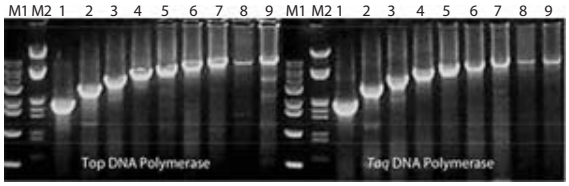

Figure 4. Long PCR amplification test of *Top* DNA Polymerase and *Taq* DNA Polymerase using Lambda DNA. 10 ng of Lambda DNA and 1 U of each DNA Polymerase used for amplification.

Lane 1: 2 kb PCR product
Lane 2: 3 kb PCR product
Lane 3: 4 kb PCR product
Lane 4: 5 kb PCR product
Lane 5: 6 kb PCR product
Lane 6: 7 kb PCR product
Lane 7: 8 kb PCR product
Lane 8: 9 kb PCR product
Lane 9: 10 kb PCR product
M1: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)
M2: Lambda DNA/*Hind* III Marker (Bioneer, Cat. No. D-1050)

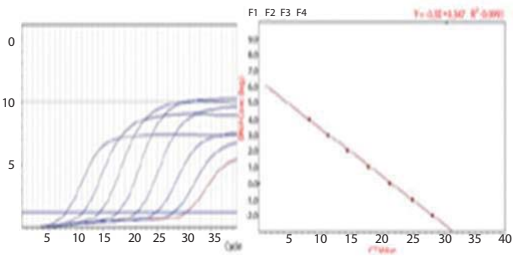

Figure 5. Sensitivity test using Real-Time qPCR with SYBR Green and *Top* DNA Polymerase. The standard curve shows a high correlation of R2 = 0.9993.

Lane 1: 100 ng lambda DNA
Lane 2: 10 ng lambda DNA
Lane 3: 1 ng lambda DNA
Lane 4: 100 pg lambda DNA
Lane 5: 10 pg lambda DNA
Lane 6: 1 pg lambda DNA
Lane 7: 100 fg lambda DNA
Lane 8: 10 pg lambda DNA

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DNA Polymerase

Top DNA Polymerase

Note : This enzyme is specifically optimized for increasing the rate of base incorporation by inactivating 5'->3' exonuclease activity. Therefore, this is not recommended to use for Real Time PCR using *Taqman*® probe, for which *AccuPower*® *DualStar*™ qPCR PreMix is recommended.

■ Ordering Information

Cat. No.	Product Description
D-3001	dNTP Mix, 10 mM, 4 tubes each 2.5 mM
E-3100	*Top* DNA Polymerase, 500 U, 10 mM dNTP, 10 x reaction buffer with 20 mM $MgCl_2$
E-3100-1	*Top* DNA Polymerase, 500 U, 10 mM dNTP, 10 x reaction buffer, 20mM $MgCl_2$
E-3100-2	*Top* DNA Polymerase, 500 U, 10 x reaction buffer with $MgCl_2$
E-3100-3	*Top* DNA Polymerase, 500 U, 10 x reaction buffer, 20 mM $MgCl_2$
E-3101	*Top* DNA Polymerase, 2,000 U, 10 mM dNTP, 10 x reaction buffer with $MgCl_2$
E-3101-1	*Top* DNA Polymerase, 2,000 U, 10 mM dNTP, 10 x reaction buffer, 20 mM $MgCl_2$
E-3101-2	*Top* DNA Polymerase, 2,000 U, 10 x reaction buffer with $MgCl_2$
E-3101-3	*Top* DNA Polymerase, 2,000 U, 10 x reaction buffer, 20 mM $MgCl_2$

HotStart DNA Polymerase

Unique enzyme-mediated Hot Start DNA Polymerase

Description

Bioneer's HotStart DNA polymerase uses a patented enzyme-mediated HotStart PCR method that, unlike most other HotStart PCR chemistries, completely releases all polymerase activity during the first denaturation step. *Top* DNA polymerase is completely inhibited by pyrophosphate at temperatures below 70℃. However, at temperatures above 70℃, a thermostable pyrophosphatase initiates pyrophosphate hydrolysis and activates the DNA polymerase. This prevents the formation of non-specific products and primer-dimers during the reaction set-up process and results in improved PCR specificity.

Features and Benefits

- Fast:
  More than three times processive than standard Taq DNA Polymerase
- High performance:
  Amplifies fragments up to 12 kb

Application

- *HotSart* PCR, PCR with complex genomic templates/low copy templates/cDNA
- Multiplex PCR
- Primer extension
- SNP typing
- Real-Time PCR using SYBR Green dye
- Multiple primer pairs and amplification of low copy template DNA

Enzyme Properties

- 5' to 3' exonuclease activity: No
- 3' to 5' exonuclease activity: No
- 3-A overhang: Yes
- Fragment size: ~12 kb

Reagents Supplied

- 10 x Reaction buffer : Tris-HCl, KCl, Pyrophosphate, pH 9.0
- 1 x Dilution bffer : 50% glycerol containing 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, pH 8.2
- dNTP Mix : 2.5 mM of each dNTP
- 20 mM $MgCl_2$

Concentration

250 U/50 µl

Storage Conditions

50% glycerol containing 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, pH 8.0

Storage Temperature

-20℃.

Unit Definition

One unit is defined at the amount of enzyme that will incorporate 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72℃

Experimental Data

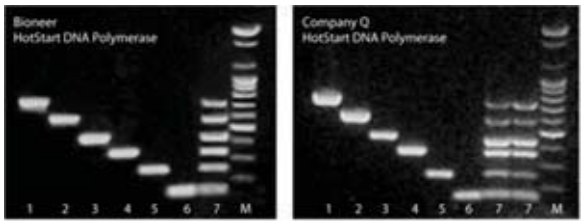


Figure 1. Multiplex PCR comparison of genomic DNA using 6 sets of primers and 2 different DNA Polymerases.

Lane 1: 750 bp fragment
Lane 2: 590 bp fragment
Lane 3: 450 bp fragment
Lane 4: 360 bp fragment
Lane 5: 260 bp fragment
Lane 6: 150 bp fragment
Lane 7: Multiplex PCR with primers used for Lane 1 – 6
Lane M: 100 bp DNA Ladder (Bioneer, Cat. No. D-1030).

01
DNA Polymerase

HotStart DNA Polymerase

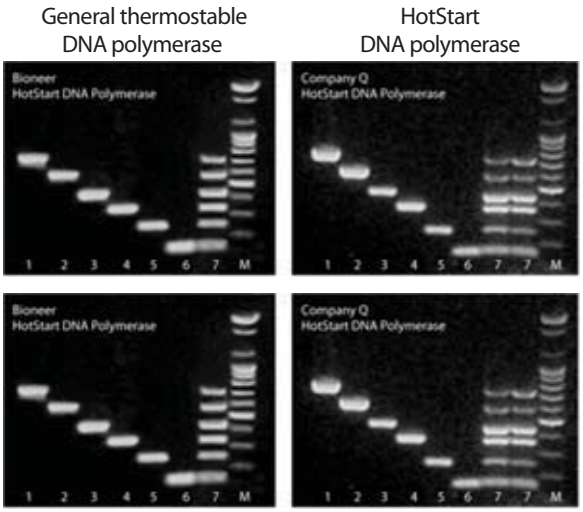


Figure 2. Real-Time PCR results (SYBR Green used)

This product is covered by a Korea Patent No. 292883 and its related pending patents in some countries of Bioneer Corporation.

Note: This enzyme is specifically optimized for increasing the rate of base incorporation by inactivating 5'->3' exonuclease activity. Therefore, this is not recommended to use for Real Time PCR using *Taqman*® probe, for which *AccuPower*® *DualStar*™ qPCR PreMix is recommended.

■ Ordering Information

Cat. No.	Product Description
E-3150	HotStart DNA Polymerase, 250 U, 10 x reaction buffer, without 20 mM $MgCl_2$, 10 mM dNTP
E-3150-1	HotStart DNA Polymerase, 250 U, 10 x reaction buffer, without 20 mM $MgCl_2$
E-3151	HotStart DNA Polymerase, 1,000 U, 10 x reaction buffer, without 20 mM $MgCl_2$, 10 mM dNTP

Pfu DNA Polymerase

Description

Pfu DNA polymerase is a thermostable DNA polymerase isolated from Pyrococcus furiosus Vc1. It catalyzes the DNA-dependent polymerization of nucleotides into duplex DNA in the 5′ ⇒ 3′ direction and exhibits 3′ ⇒ 5′ exonuclease (proofreading) activity. Pfu DNA polymerase is the ideal choice for a variety of techniques requiring high-fidelity DNA synthesis by PCR reaction.

Features and Benefits

- High fidelity PCR:
  3′ to 5′ exonuclease (proofreading) activity
- Thermostability:
  Retaining 94 – 99 % of its thermostable activity after 1 hour at 95℃
- Terminal Transferase Activity:
  Devoid of terminal transferase activity and generates blunt-ended PCR products

Application

- Gene synthesis
- High fidelity PCR or Primer extention.
- Blunt-end PCR Cloning or mutagenesis requesting high fidelity
- Site directed mutagenesis.

Enzyme Properties

- 5' to 3' exonuclease activity: No
- 3' to 5' exonuclease activity: Yes
- 3′ – A overhang: No
- Fragment size: ~ 10 kb

Reagents Supplied

- 10 x Reaction Buffer: $MgSO_4$, Tris-HCl, KCl, $(NH_4)_2SO_4$, Acetylated BSA pH 8.8
- dNTP Mix: 2.5 mM of each dNTP (optional)

Concentration

250 U/100 µl

Storage Temperature

-20℃.

Unit Definition

One unit is defined at the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 72℃.

Experimental Data

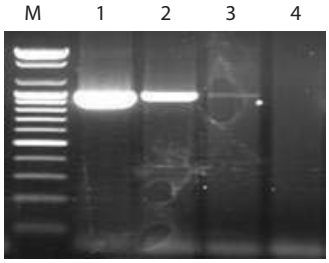


Figure 1. Human DNA was amplified using 2.5 units of enzyme in 50 ul reaction volume

Lane 1: 20 ng Lane 2: 2 ng Lane 3: 200 pg Lane 4: 20 pg
M: 100 bp DNA ladder(Bioneer, Cat. No. D-1030)

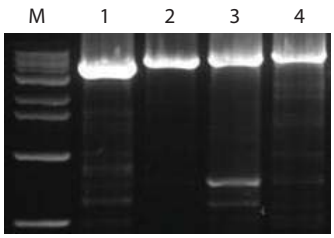


Figure 2. PCR test of Long size Lambda DNA with *Pfu* DNA polymerase.

Lane 1: Lambda DNA 5 Kb, Lane 2: Lambda DNA 6 Kb,
Lane 3: Lambda DNA 7 Kb,
Lane 4: Lambda DNA 8 Kb, M: 1 Kb DNA ladder
(Bioneer, Cat No.: D-1040)

01
DNA Polymerase

Pfu DNA Polymerase

■ Ordering Information

Cat. No.	Product Description
E-2015	*Pfu* DNA Polymerase, 250 U, 10 x reaction buffer, without dNTPs
E-2016	*Pfu* DNA Polymerase, 1,000 U, 10 x reaction buffer, without dNTPs

01
DNA Polymerase

Taq DNA Polymerase

Description

Taq DNA Polymerase is a thermostable DNA polymerase that catalyzes the polymerization of nucleotides into duplex DNA in the 5′ to 3′ direction. Bioneer's *Taq* DNA Polymerase is isolated from recombinant *E.coli* strain containing the DNA polymerase gene from Thermus aquaticus YT1. It exhibits its highest activity at pH 9.0 and 72°C.

Features and Benefits

- Improved yield & sensitivity:
  Perform high yield and high sensitive PCR using Bioneer *Taq* DNA polymerase.
- Versatility:
  Use for a wide range of DNA amplifications including Real-Time PCR using *TaqMan* probe or SYBR Green.
- Robust performance:
  Optimized reaction buffer enhances PCR performance.

Application

Routine PCR
SYBR-Green-based qPCR
Dual-labeled probe based qPCR
Primer extension
TA cloning
Gene sequencing
Gene expression profiling
Microbial & viral pathogen detection

Enzyme Properties

- Concentration: 5 U/ul
- 5' to 3' exonuclease activity: Yes
- 3' to 5' exonuclease activity: No
- 3′-A overhang: Yes
- Nuclease contamination: No
- Extension rate: 3-10 kb/min depending on template complexity
- Fragment Size: Up to 10 kb

Reagents Supplied

- 10 x Reaction buffer with (or without) $MgCl_2$: 100 mM Tris-HCl, 400 mM KCl, 15 mM $MgCl_2$, pH 9.0
- Dilution buffer: 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, Stablizers, 50 % Glycerol, pH 8.0
- dNTP Mix: 2.5 mM of each dNTP

Concentration

500 units (5 U/ul)

Storage Conditions

20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, Stablizers, 50 % Glycerol pH 8.0

Storage Temperature

-20℃.

Unit Definition

One unit is defined at the amount of enzyme that will incorporate 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72℃.

Experimental Data

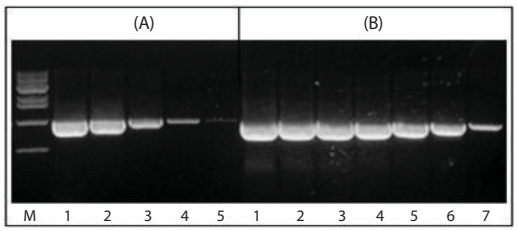


Figure 1. Enzyme activity of *Taq* DNA polymerase was tested on human genomic DNA (A) and Lambda DNA (B) template using 1 U *Taq* DNA polymerase. Each template DNA was serially diluted by ten-folds, with different ranges.

Lane 1: 100 ng Template DNA
Lane 2: 10 ng Template DNA
Lane 3: 1 ng Template DNA
Lane 4: 100 pg Template DNA
Lane 5: 10 pg Template DNA
Lane 6: 1 pg Template DNA
Lane 7: 100 fg Template DNA
M: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

01 DNA Polymerase

Taq DNA Polymerase

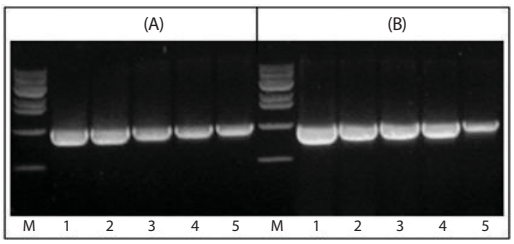


Figure 2. Enzyme activity test of *Taq* DNA polymerase. *Taq* DNA polymerase was serially diluted from 5 U to 0.25 U and was used to amplify Human genomic DNA (A) and Lambda DNA (B).

Lane 1: 5 U of *Taq* DNA polymerase
Lane 2: 1 U of *Taq* DNA polymerase
Lane 3: 0.5 U of *Taq* DNA polymerase
Lane 4: 0.33 U of *Taq* DNA polymerase
Lane 5: 0.25 U of *Taq* DNA polymerase
M: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

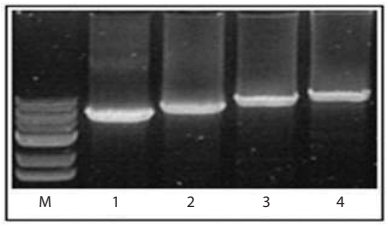


Figure 3. Enzyme activity test of *Taq* DNA polymerase. *Taq* DNA polymerase was serially diluted from 5 U to 0.25 U and was used to amplify Human genomic DNA (A) and Lambda DNA (B).

Lane 1: 5 kb PCR product
Lane 2: 6 kb PCR product
Lane 3: 7 kb PCR product
Lane 4: 8 kb PCR product
M: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

■ Ordering Information

Cat. No.	Product Description
D-3001	dNTP Mix, 10 mM, each 2.5 mM
E-2011	*Taq* DNA Polymerase, 500 U, 10 mM dNTP, 10 x reaction buffer with $MgCl_2$
E-2011-1	*Taq* DNA Polymerase, 500 U, 10 mM dNTP, 10 x reaction buffer, 20mM $MgCl_2$
E-2011-2	*Taq* DNA Polymerase, 500 U, 10 x reaction buffer with $MgCl_2$
E-2011-3	*Taq* DNA Polymerase, 500 U, 10 x reaction buffer, 20 mM $MgCl_2$
E-2013	*Taq* DNA Polymerase, 2,000 U, 10 mM dNTP, 10 x reaction buffer with $MgCl_2$
E-2013-1	*Taq* DNA Polymerase, 2,000 U, 10 mM dNTP, 10 x reaction buffer, 20 mM $MgCl_2$
E-2013-2	*Taq* DNA Polymerase, 2,000 U, 10 x reaction buffer with $MgCl_2$
E-2013-3	*Taq* DNA Polymerase, 2,000 U, 10 x reaction buffer, 20 mM $MgCl_2$

01
DNA Polymerase

HotStart *Taq* DNA Polymerase

Description

HotStart *Taq* DNA Polymerase is designed to increase specificity and sensitivity in PCR. HotStart *Taq* DNA polymerase is inhibited at temperatures lower than 70℃, but is fully activated after the first denaturation step. This prevents the formation of mis-primed products and primer-dimers during the reaction setup process, resulting in improved PCR specificity.

Features and Benefits

- Maximized specificity:
  Virtually eliminates non-specific amplification. Ideal for multiplex PCR with 2-6 amplicons.
- Improved sensitivity:
  Excellent for PCR using low copy number targets
- TA cloning compatible:
  PCR products amplified with HotStart Taq DNA polymerase have 3′-A overhang and can be used for TA cloning.
- Versatility:
  HotStart Taq DNA Polymerase is ideal for a wide range of PCR applications.

Application

- Multiplex PCR
- HotStart PCR
- Routine PCR
- SYBR-Green-based qPCR
- Dual-labeled probe based qPCR
- Real-Time quantification of DNA and cDNA targets using SYBR Green dye
- Primer extension
- TA cloning
- Gene sequencing
- SNP

Enzyme Properties

- Concentration: 5 U/μl
- 5' to 3' exonuclease activity: Yes
- 3' to 5' exonuclease activity: No
- 3′–A overhang: Yes
- Nuclease contamination: Certified DNase and RNase free
- Extension rate: 3–10 kb/minute depending on template complexity
- Fragment Size: Up to 10 kb

Reagents Supplied

- 10X Reaction Buffer: Contains Tris-HCl, KCl, 15 mM $MgCl_2$, pH 9.0
- Dilution Buffer: 20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, Stabilizers, 50 % Glycerol, pH 8.0
- dNTPs mixture: 10 mM, each dNTP 2.5 mM

Concentration

250 U/50 ul

Storage Conditions

20 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, 100 mM KCl, Stabilizers, 50 % Glycerol, pH 8.0

Storage Temperature

-20℃.

Unit Definition

One unit is defined at the amount of enzyme that will incorporate 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72℃.

Experimental Data

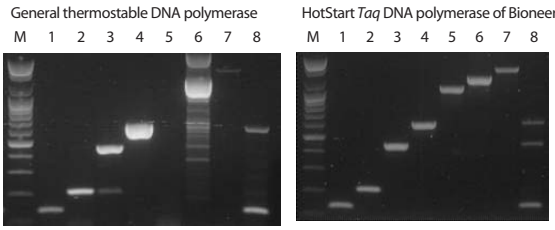


Figure 1. Specificity comparison between standard *Taq* and HotStart *Taq* DNA polymerase Single and Multiples PCR results in human genomic DNA p53 Gene amplification.

Lane 1: 139 bp Lane 2: 211 bp
Lane 3: 447 bp Lane 4: 618 bp
Lane 5: 1,082 bp Lane 6: 1,296 bp
Lane 7: 1,561 bp
Lane 8: Multiplex PCR(139 bp, 447 bp, 618 bp)
M: 100 bp DNA Ladder (Bioneer, Cat. No D-1030)

01
DNA Polymerase

HotStart *Taq* DNA Polymerase

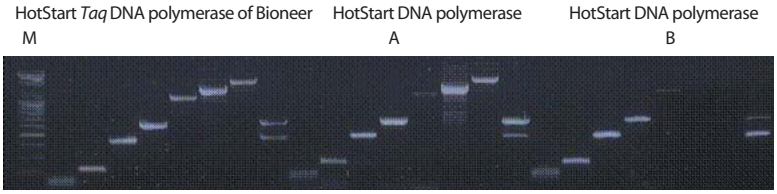


Figure 2. Performance comparison between HotStart *Taq* Polymerase and other supplier.

M : 100 bp DNA Ladder (Bioneer, Cat. No. D-1040)
A : competitor A B : competitor B

Ordering Information

Cat. No.	Product Description
E-2017	HotStart *Taq* DNA Polymerase, 250 U, 10 x reaction buffer with 20 mM $MgCl_2$, 10 mM dNTP
E-2017-1	HotStart *Taq* DNA Polymerase, 1,000 U, 10 x reaction buffer with 20 mM $MgCl_2$, 10 mM dNTP
E-2017-2	HotStart *Taq* DNA Polymerase, 500 U, 10 x reaction buffer with 20 mM $MgCl_2$, 10 mM dNTP
E-2017-3	HotStart *Taq* DNA Polymerase, 250 U, 10 x reaction buffer with 20 mM $MgCl_2$
E-2017-4	HotStart *Taq* DNA Polymerase, 1,000 U, 10 x reaction buffer with 20 mM $MgCl_2$

01
DNA Polymerase

ProFi Taq DNA polymerase

Description

ProFi Taq DNA polymerase is a unique recombinant *Taq* DNA polymerase that offers enhanced amplification efficiency for PCR. *ProFi Taq* DNA polymerase provides long-range PCR and higher fidelity than conventional *Taq* DNA polymerase. This enzyme is applicable to any template DNA, and especially effective in amplifying large genomic DNA fragments up to 20 kb. *ProFi Taq* DNA polymerase provides accurate long-range amplification of standard and complex templates and amplification of low-copy target, and is highly suitable for all PCR applications.

Features and Benefits

- Ideal for long PCR:
  Amplify fragments up to 20 kb from Human genomic DNA and 30 kb from Lambda DNA.
- Versatility:
  Suitable for all PCR applications and especially effective in long-range amplification and amplification of low-copy target.
- Reproducibility:
  Bioneer's strict quality controlled production system ensures that your results will be reproducible experiment after experiment.

Applications

- Primer extension
- long-range amplification from genomic DNA
- High amplification efficiency
- Excellent performance on difficult templates
- Amplification of low-copy targets
- High yield and high sensitivity PCR

Enzyme Properties

- Concentration: 5 U/μl
- 5′ to 3′ exonuclease: Yes
- 3′ to 5′ exonuclease: No
- 3′ - A overhang: Yes
- PCR product size: ~ 30 kb

Concentration

250 U/50 μl

Experimental data

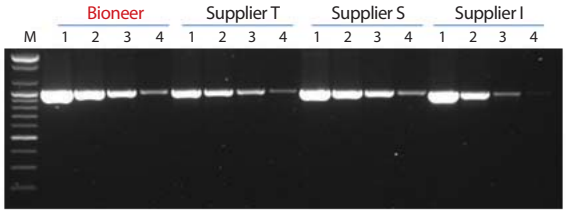


Figure 1. Comparison of PCR amplification efficiency between *ProFi Taq* DNA polymerase from Bioneer and other suppliers' DNA polymerase.
The cycling conditions for *ProFi Taq* DNA polymerase were 95°C for 5 min, 30 cycles of 95°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec. PCR reaction using other suppliers' DNA polymerase were performed according to each supplier's protocol.

Target: human Insulin receptor gene.
Lane 1: 10 ng of human genomic DNA
Lane 2: 1 ng of human genomic DNA
Lane 3: 100 pg of human genomic DNA
Lane 4: 10 pg of human genomic DNA
Lane M: 100 bp DNA Ladder(Bioneer, Cat. No. D-1030)

01
DNA Polymerase

ProFi Taq DNA polymerase

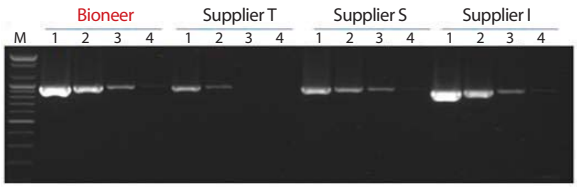


Figure 2. Comparison of PCR amplification efficiency between *ProFi Taq* DNA polymerase from Bioneer and other suppliers DNA polymerase.

cDNA synthesized from 10-fold serial-diluted human total RNA from 10 ng to 10 pg using *AccuPower*® *RocketScript*™ Cycle RT PreMix.(Bioneer, Cat. No K-2201) wase used as a template for PCR amplification. The cycling conditions for *ProFi Taq* DNA polymerase were 95°C for 5 min, 33 cycles of 95°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec. PCR reactions using other suppliers DNA polymerase were performed according to each supplier's protocol.

Target : human GAPDH gene.
Lane 1: 10 ng of human total cDN
Lane 2: 1 ng of human total cDNA
Lane 3: 100 pg of human total cDNA
Lane 4: 10 pg of human total cDNA
Lane M: 100 bp DNA Ladder(Bioneer, Cat. No. D-1030)

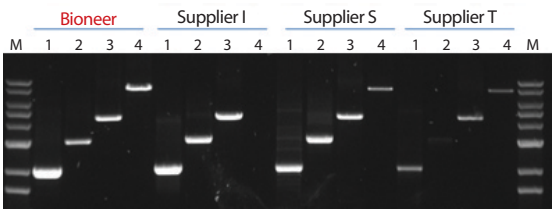


Figure 3. Comparison of PCR amplification of long targets between *ProFi Taq* DNA polymerase from Bioneer and other suppliers DNA polymerase.

The cycling conditions for *ProFi Taq* DNA polymerase were 95} ℃ for 5 min, 30 cycles of 95 ℃ for 20 sec, 65 ℃ for 20 sec and 68 ℃ for 4 min. PCR reactions using other suppliers DNA polymerase were performed according to each supplier's protocol.

Lane 1: 2 kb fragment (human tumor protein p53 gene)
Lane 2: 3 kb fragment (human tumor protein p53 gene)
Lane 3: 4.5kb fragment (human DNA cross-link repair 1A gene)
Lane 4: 8 kb fragment (human hemoglobin epsilon 1 gene)
Lane M1: Lambda/*Hind* III marker (Bioneer, Cat. No. D-1050)
Lane M2: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

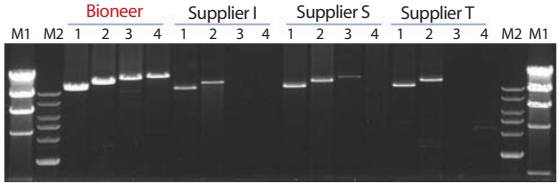


Figure 4. Comparison of PCR amplification of long targets between *ProFi Taq* DNA polymerase from Bioneer and other suppliers' DNA polymerase.

The cycling conditions for *ProFi Taq* DNA polymerase were 95℃ for 5 min, 32 cycles of 95℃ for 20 sec and 68℃ for 15 min. PCR reactions using other suppliers' DNA polymerase were performed according to each supplier's protocol. Human genomic DNA was used as a template for PCR amplification.

Lane 1: 11 kb fragment
Lane 2: 13.5 kb fragment
Lane 3: 17.6 kb fragment
Lane 4: 21.4 kb fragment
Lane M1: Lambda/*Hind* III marker (Bioneer, Cat. No. D-1050)
Lane M2: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

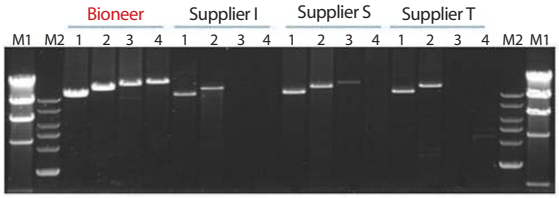


Figure 5. Comparison of PCR amplification of long targets between *ProFi Taq* DNA polymerase from Bioneer ad other suppliers' DNA polymerase.

The cycling conditions for *ProFi Taq* DNA polymerase were 95 ℃ for 5 min, 32 cycles of 95 ℃ for 20 sec, 65 ℃ for40 sec, and 68 ℃ for 20 min. PCR reactions using other suppliers' DNA polymerase were performed according to each supplier's protocol. Lambda DNA was used as a template for PCR amplification.

Lane 1: 15 kb fragment
Lane 2: 20 kb fragment
Lane 3: 25 kb fragment
Lane 4: 30 kb fragment
Lane M1: Lambda/*Hind* III marker (Bioneer, Cat. No. D-1050)
Lane M2: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040

ProFi Taq DNA polymerase

Ordering Information

Cat. No.	Product Description
E-2201	*ProFi Taq* DNA Polymerase 250 units, 10 mM dNTPs, 10 X reaction buffer with $MgCl_2$
E-2202	*ProFi Taq* DNA Polymerase 250 units, 10 mM dNTPs, 10 X reaction buffer without $MgCl_2$, 20 mM $MgCl_2$
E-2203	*ProFi Taq* DNA Polymerase 250 units, 10 X reaction buffer with $MgCl_2$
E-2204	*ProFi Taq* DNA Polymerase 250 units, 10 X reaction buffer without $MgCl_2$, 20 mM $MgCl_2$
E-2205	*ProFi Taq* DNA Polymerase 1000 units, 10 mM dNTPs, 10 X reaction buffer with $MgCl_2$
E-2206	*ProFi Taq* DNA Polymerase 1000 units, 10 mM dNTPs, 10 X reaction buffer without $MgCl_2$, 20 mM $MgCl_2$
E-2207	*ProFi Taq* DNA Polymerase 1000 units, 10 X reaction buffer with $MgCl_2$
E-2208	*ProFi Taq* DNA Polymerase 1000 units, 10 X reaction buffer without $MgCl_2$, 20 mM $MgCl_2$

02 Reverse Transcriptase

Reverse Transcriptase

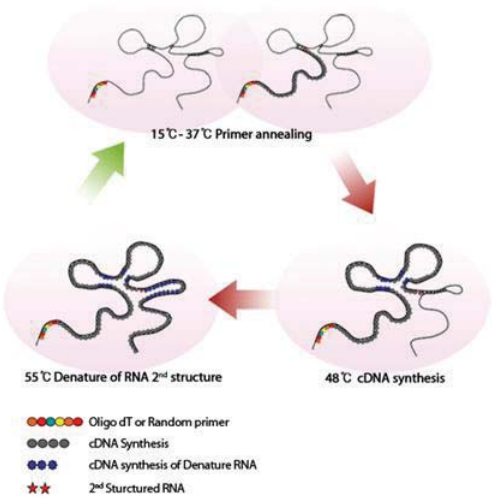
CycleScript Reverse Transcriptase

Description

Get more cDNA in less time with CycleScript Reverse Transcriptase. CycleScript is a versatile reverse transcriptase - applicable to both conventional Reverse Transcription and Cyclic Reverse Transcription (Cyclic RT, patent pending – for cDNA amplification). It features high activity across a wide range of temperatures from 37 to 55°C therefore, reverse transcription is carried out like PCR. The Cyclic RT reaction is composed of the following steps: 1st incubation at 15 - 40°C for primer annealing, heating up to 42 - 48°C for extension, and finally incubation at 50 - 55°C for denaturation of the secondary structure of the RNA (optional). Bioneer's novel Cyclic RT system offers homogeneous cDNA synthesis, with a high yield of cDNA up to 9 kb.

Features and Benefits

- Broad range of working temperatures:
  For GC rich RNAs or RNAs with significant secondary structure
- Sensitivity:
  Even the rarest transcript can be reliably made into cDNA
- High yield of cDNA:
  For genes up to 9 kb within 10 minutes
- RNase, DNase, and Proteinase-free:
  Ensures the integrity of your samples



Application

- First-strand synthesis of cDNA from RNA molecules
- RT-PCR
- Random priming reaction
- Library construction
- Probe labeling
- mRNA 5′ end mapping by primer extension analysis

Enzyme Properties

- 5' to 3' exonuclease activity: No
- 3' to 5' exonuclease activity: No
- 3′ – A overhang: Yes
- Strand displacement: Yes
- Fragment size: Up to 9 kb

Reagents Supplied

- 5 x Reaction Buffer: 50 mM Tris-HCl, 250 mM KCl, 10 mM $MgCl_2$, pH 8.1
- 100 mM DTT
- dNTP Mix: 2.5 mM of each dNTP

02
Reverse Transcriptase

CycleScript Reverse Transcriptase

Concentration

- 10,000 U/50 µl

Storage Conditions

50% glycerol containing 20 mM Tris-Cl, 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 % IGEPAL CA-630

Storage Temperature

-20℃.

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmole of dTTP into acid-precipitable material in 10 minutes at 37℃ using poly (A)oligo (dT) as template primer.

Principles

1st strand cDNA synthesis [20 uL volume]

Step 1 Mix template and primer

Total RNA (0.1~1 ug) or mRNA (5 ng ~ 100 ng)
+
add Oligo dT or Random hexamer(50 ~ 200 pmole)

* Denature RNA and primer for 10 min at 65°...
* Immediately cool on ice
(* Note. Optional when CTRT is performed)

Step 2 All components mix

5X CycleScript reaction buffer	4 uL
100mMDTT	2 uL
10mM dNTP (variable volume)	2 uL
Nase inhibitor	20 units
CycleScript(200unit/ul)	1 uL
DEPC-DW	variable
Total (Step1 + Step 2)	20 uL

[Temperature]

[Option 1:CTRT]

Temperature	Time	
15 ~ 37℃	30 sec	Repeat 12 times
42 ~ 48℃	4 min	Deactivation
95℃	5 min	Deactivation

[Option 2:FTRT]

Temperature	Time
37 ~ 55℃	1 hr
55℃(Optional)	30 sec
90℃	5 min

Experimental Data

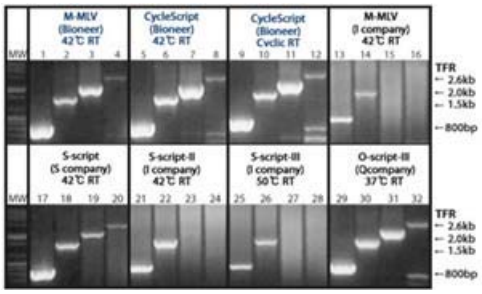


Figure 1. Comparison of transferrin receptor gene amplification with different reverse transcriptases.
700 ng of total RNA was used for reverse transcription and the same amount of amplified products were used for electrophoresis.

Lane 1 - 4: TFR (Transferrin receptor gene) amplified with *MMLV*
Lane 5 - 8: TFR amplified with CycleScript
Lane 9 - 12: TFR amplified with CycleScript
Lane 13 - 16: TFR amplified with *MMLV* from Company IA
Lane 17 - 20: TFR amplified with S-script from Company S
Lane 21 - 24: TFR amplified with S-script II from Company I
Lane 25 - 28: TFR amplified with S-script III from Company I
Lane 29 - 32: TFR amplified with O-script from Company Q
Lane MW: 100 bp Plus DNA Ladder (Bioneer, Cat. No. D-1035)

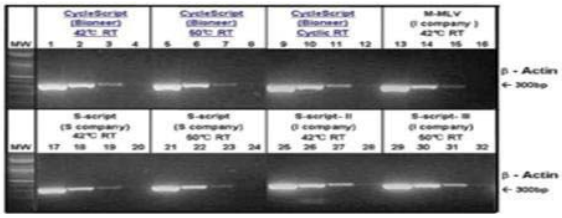


Figure 2. Comparison of β-actin gene amplification with different reverse transcriptases.
Each 10 ng, 1 ng, 100 pg, and 10 pg of total RNA was used for reverse transcription and the same amount of amplified products were used for electrophoresis.

Lane 1 - 4: beta-actin amplified with CycleScript
Lane 5 - 8: beta-actin amplified with CycleScript
Lane 9 - 12: beta-actin amplified with CycleScript
Lane 13 - 16: beta-actin amplified with MMLV from Company I
Lane 17 - 20: beta-actin amplified with S-script from Company S
Lane 21 - 24: beta-actin amplified with S-script from Company S
Lane 25 - 28: beta-actin amplified with S-script from Company I
Lane 29 - 32: beta-actin amplified with S-script from Company I

CycleScript Reverse Transcriptase

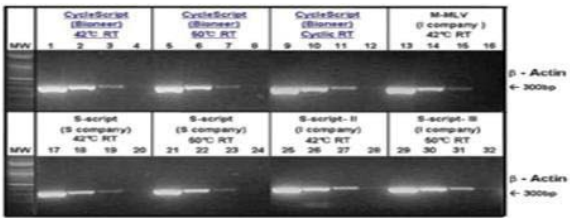


Figure 3. Comparison of GAPDH gene amplification with different reverse transcriptases.

Each 10 ng, 1 ng, 100 pg, and 10 pg of total RNA was used for reverse transcription and the same amount of amplified products were used for electrophoresis.

Lane 1-4: GAPDH amplified with CycleScript
Lane 5-8: GAPDH amplified with CycleScript
Lane 9-12: GAPDH amplified with CycleScript
Lane 13-16: GAPDH amplified with MMLV from Company I
Lane 17-20: GAPDH amplified with S-script from Company S
Lane 21-24: GAPDH amplified with S-script from Company S
Lane 25-28: GAPDH amplified with S-script from Company I
Lane 29-32: GAPDH amplified with S-script from Company I

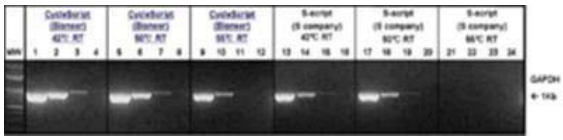


Figure 4. Working temperature comparison of different reverse transcriptases.
Each 10 ng, 1 ng, 100 pg, and 10 pg of total RNA was used for reverse transcription and the same amount of amplified products were used for electrophoresis.

Lane 1-4: GAPDH amplified with CycleScript
Lane 5-8: GAPDH amplified with CycleScript
Lane 9-12: GAPDH amplified with CycleScript
Lane 13-16: GAPDH amplified with S-script from company S
Lane 17-20: GAPDH amplified with S-script from company S
Lane 21-24: GAPDH amplified with S-script from company S

Ordering Information

Cat. No.	Product Description
E-3131	CycleScript Reverse Transcriptase, 10,000 U, 5 x Reaction Buffer, 100 mM DTT, 10 mM dNTP
E-3132	CycleScript Reverse Transcriptase, 50,000 U, 5 x Reaction Buffer, 100 mM DTT, 10 mM dNTP

02 Reverse Transcriptase

MMLV Reverse Transcriptase

Description

Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase is an RNA-dependent DNA polymerase. This enzyme is able to use an RNA molecule as a template and synthesize a double-stranded DNA. *M-MLV* Reverse Transcriptase is isolated from an *E.coli* strain containing a recombinant clone. It is ideal for use of first-strand synthesis cDNA from RNA molecules and for cDNA synthesis for Reverse Transcriptase PCR and RT-qPCR.

Source : *M-MLV* Reverse Transcriptase is isolated from an *E.coli* strain containing a recombinant clone.

Features and Benefits

- Optimized 5X buffer:
  For full length of cDNA synthesis
- Full length cDNA:
  For genes up to 9 kb
- RNase, DNase and Proteinase-free:
  Ensures the integrity of your samples

Application

First strand cDNA synthesis from RNA, RT-PCR, and qRT-PCR

Enzyme Properties

- 5' to 3' exonuclease activity: No
- 3' to 5' exonuclease activity: No
- 3' – A overhang: No
- Strand displacement: Yes
- Fragment size: Up to 9 kb

Reagents Supplied

- 5 x Reaction Buffer: 50 mM Tris-HCl, 250 mM KCl, 10 mM $MgCl_2$, pH 8.1
- 100 mM DTT
- dNTP Mix: 2.5 mM of each dNTP

Concentration

10,000 U/50 ul

Storage Conditions

50% glycerol containing 20 mM Tris-Cl (pH7.6), 150 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 % IGEPAL CA-630

Storage Temperature

-20℃ .

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmole of dTTP into acid-precipitable material in 10 minutes at 37℃ using poly (A)oligo (dT) as template primer.

Ordering Information

Cat. No.	Product Description
E-3121	*MMLV* Reverse Transcriptase, 10,000 U, 5 x Reaction Buffer, 100 mM DTT
E-3122	*MMLV* Reverse Transcriptase, 50,000 U, 5 x Reaction Buffer, 100 mM DTT

02
Reverse Transcriptase

RocketScript™ Reverse Transcriptase

Description

RocketScript™ is Bioneer's exclusive *M-MLV* based thermostable reverse transcriptase (RTase).

Native *M-MLV* RTase has maximum activity at relatively low temperatures (42°C), however it causes several problems in reverse transcription of RNA molecules with complex secondary structure. *RocketScript*™ has thermostable activity (42 – 70°C), allowing efficient cDNA synthesis from complex secondary structure RNA.

Features and Benefits

- **Thermostable Activity**: Native M-MLV reverse transcriptase has low thermostable activity, therefore restricting reverse transcription reactions to relatively low temperatures (42°C). This attribute prevents RNA molecules containing many stems and loops (complex secondary structures) from being efficiently transcribed. To resolve this shortcoming, Bioneer has utilized synthetic biotechnology to develop a RTase that is active even at high temperatures of 60°C and above. By removing the traditional reaction temperature limit of 42°C, you are now able to choose your reaction temperature from 42 – 70°C and optimize your cDNA synthesis experiments.

- **Enhanced Performance**: While engineering the enzyme, the researchers at Bioneer have gone ahead and engineered robust performance into RocketScript. You can now confidently perform experiments with target lengths long and short, or input RNA concentrations low and high knowing that if the RNA is in the sample, it will be accurately represented in the cDNA.

- **Ease-of-use**: All components necessary for cDNA synthesis including thermostable RTase and RNase inhibitor are included in the product for ease-of-use. All you need is source RNA and primers, and preparation for your reverse transcription reaction is complete.

- **Reproducibility**: Each batch is produced under strict quality controls. Errors that commonly occur during mass production are eliminated by ding individual packaging. Bioneer's current batch processing system allows for the production of more accurate and reproducible end-product yield.

Application

- Gene synthesis
- First-strand synthesis of cDNA from RNA molecules (Reverse Transcription)
- RT-PCR and RT-qPCR
- Random priming reactions
- Library construction
- Probe labeling
- mRNA 5-end mapping by primer extension analysis
- Real-Time PCR

Specifications

- 5' to 3' exonuclease: No
- 3' to 5' exonuclease: No
- 3' - A overhang: No
- Fragment size: Up to 10 kb

Concentration

10,000 U/50 µl

Storage Temperature

-20℃.

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmole of dTTP into acid-precipitable material in 10 minutes at 37℃ using poly (A)oligo (dT) as template primer.

02 Reverse Transcriptase

RocketScript™ Reverse Transcriptase

■ Experimental data

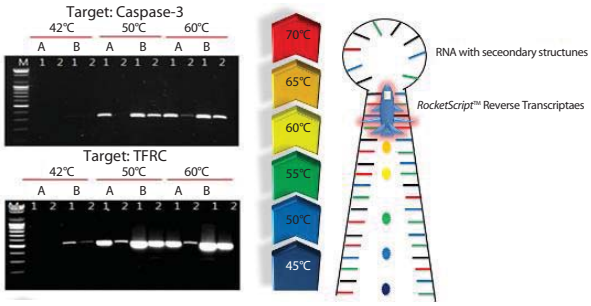
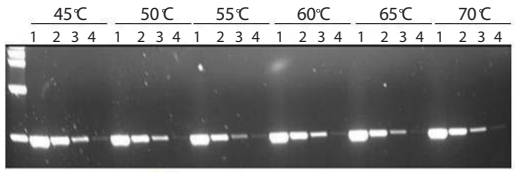


Figure 1. Complex RNA amplification

Complex RNA amplification results of *RocketScript*™ Reverse Transcriptase compared with that of conventional RTase
Reverse transcription condition : conventional 42℃ / 50℃ / 60℃ 1hr, deactivation 95℃ 5min, this Product shows thermal stability.

Lane A: *M-MLV*™ Reverse Transcriptase
Lane B: *RocketScript*™ Reverse Transcriptase
Lane 1: 100 ng human total RNA from HeLa cell
Lane 2: 10 ng human total RNA from HeLa cell

Thermostable Acivity of Rocketscript RTase



Thermostable Activity of competitor i's reverse transcriptase

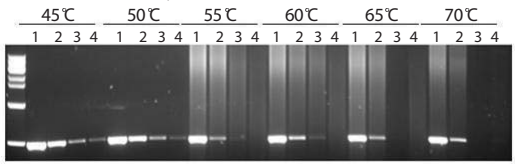


Figure 2. Thermostable Stability Check

Amplification results of *RocketScript*™ Reverse Transcriptase using Myc compared with supplier I
Reverse transcription condition : Incubation at each temperature 45, /50,/ 55, /60, /65, /70℃ for 1 hr, deactivation at 95℃ for 5 min
Primer set: human myc 495bp set

Lane 1: 100 ng hman total RNA from HeLa cell
Lane 2: 10 ng hman total RNA from HeLa cell
Lane 3: 1 ng hman total RNA from HeLa cell
Lane 4: 100 pg hman total RNA from HeLa cell
Lane M: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

Figure 3. Comparison of amplification quality between RocketScript RTase and competitor RTases

Sensitivity test. Target gene expression Level.

Lane 1: 100 ng human total RNA from HeLa cell
Lane 2: 10 ng human total RNA from HeLa cell
Lane 3: 1 ng human total RNA from HeLa cell
Lane 4: 100 pg human total RNA from HeLa cell
Lane M: 1 kb DNA Ladder (Bioneer, Cat. No. D-1040)

■ Ordering Information

Cat. No.	Product Description
E-3141	*RocketScript*™ Reverse Transcriptase, 10,000 U (50 rxns)
E-3142	*RocketScript*™ Reverse Transcriptase, 50,000 U (250 rxns)

03 DNA Ligase

DNA Ligase

03
DNA Ligase

T4 DNA Ligase

Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will ligate DNA from blunt-end and cohesive-end termini (Including sticky ends for TA Cloning) as well as repair single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids. T4 DNA Ligase is isolated from a recombinant E. coli strain.

Features and Benefits

- High Speed: DNA ligations in 5 minutes for cohesive end and in 10 minutes for blunt end DNA at 25°C
- Flexibility: Suitable for all common DNA ligations
- Reproducibility: Batch manufacturing under strict ISO 9001 quality control system.

Application

- Blunt or cohesive-end ligation
- Repair of nicks in double-stranded nucleic acids

Enzyme Properties

Heat Inactivation: 70°C for 10 minutes

Reagents Supplied

10 x Reaction Buffer: 500 mM Tris-HCl (pH 7.8), 100 mM $MgCl_2$, 50 mM DTT, 10 mM ATP, 25 ug/ml BSA

Concentration

200 U/µl

Storage Conditions

50% glycerol containing 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM EDTA, 10 mM 2-Mercaptoethanol

Storage Temperature

-20℃.

Unit Definition

0.01 Weiss unit of enzyme is defined as the amount of enzyme required to give 90% ligation of *Hind* III fragments of lambda DNA in 30 minutes, at 16℃ in 20 ul of the assay mixture.

Experimental Data

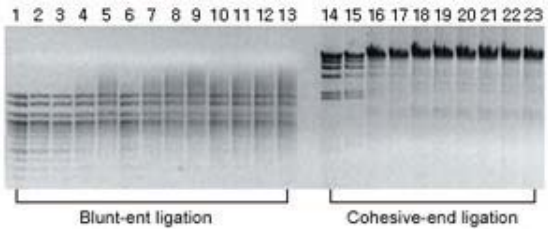


Figure 1. 0.01 Weiss unit of enzyme is defined as the amount of enzyme required to give 90% ligation of Hind III fragments of lambda DNA in 30 minutes, at 16°ΔC in 20 •ìl of the assay mixture

Lane 1: DNA fragment (digested with *EcoR* V)
Lane 2, 3, 4, 5: T4 DNA Ligase 1 U, 16°C, 10, 20, 30 and 60 minutes
Lane 6, 7, 8, 9: T4 DNA Ligase 1 U, 25°C, 10, 20, 30 and 60 minutes
Lane 10, 11, 12,13: T4 DNA Ligase 1 U, 37°C, 10, 20, 30 and 60 minutes
Lane 14: Lambda DNA (digested with *Hind* III)
Lane 15, 16, 17: T4 DNA Ligase 1 U, 16°C, 10, 20 and 30 minutes
Lane 18, 19, 20: T4 DNA Ligase 1 U, 25°C, 10, 20 and 30 minutes
Lane 21, 22, 23: T4 DNA Ligase 1 U, 37°C, 10, 20 and 30 minutes

Ordering Information

Cat. No.	Product Description
E-3061	T4 DNA Ligase, 20, 000 µ, 1 tube
E-3062	T4 DNA Ligase, 100,000 µ, 100 U x 5 tubes

AccuRapid™ DNA Ligation Kit

Description

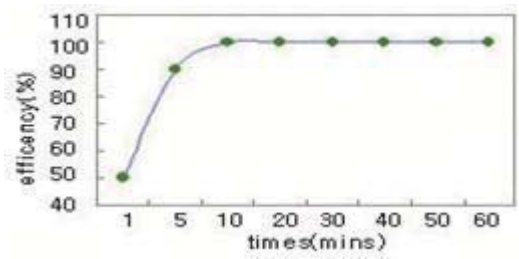
AccuRapid™ DNA Ligation Kit is a product can ligate cohesive-ended DNA fragment and blunt-ended DNA fragment within 5 minutes that reaction optimal temperature is from 15℃ to 25℃. The optimized Buffer of this product can give highly efficient ligation result through cohesive-end and blunt-end DNA fragment reaction.The Ligated product is possible to use directly into transformation.

Cat. No. K-7101 Contents: For 50 ligations
50 unit(1 unit/ul) *AccuRapid*™ DNA Ligase(from T4 bacteriophage)
0.5 mL 2x *AccuRapid*™ DNA ligation buffer

Cat. No. K-7102 Contents: For 150 ligations
50 unit x 3 (1 unit/ul) *AccuRapid*™ DNA Ligase(from T4 bacteriophage)
0.5 mL x 3 2x *AccuRapid*™ DNA ligation buffer

Quality Control Assay

The Kit is pre-tested in a ligation according to the standard protocol; 500 ng of blunt-ended 500 bp insert DNA was ligated to 100 ng of pUC19 DNA digested with Sma I. Vector DNA fragments should most preferably be dephosphory lated to minimize self-circularization. Yield of white colonies after transformation is over 90%.



Quality Assurance:

Nuclease activity is not detected after incubation of 1 μg of substrate DNA with 10 units of *AccuRapid*™ DNA Ligase in 20 μl reaction volume with the supplied Reaction buffer for 18 hrs at 37°C.

References

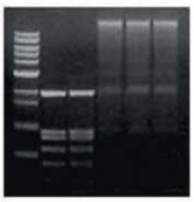
- Feiffer, B. H. and Zimmerman, S,B., Nucleic Acids Research, 11, 7853 ~ 7871, 1983.
- Hayashi., Nakazawa, M., Ishizaki, Y., Hiraoka, N., and Obayashi, A., Nucleic Acids Research, 13, 7979 ~ 7992, 1985.

Storage Condition

tore at -20 ℃.

Experimental Data

1 2 3 4 5 6



Reaction condition

Blunt-end DNA fragments	3 ul (300 ng)
2X *AccuRapid*™ DNA Ligation Buffer	10 ul
AccuRapid™ DNA Ligase	1 ul (1 unit)
Sterilized D.W	6 ul

Figure 1. Ligation test of reaction temperatures
Lane 1: 1 Kb DNA Ladder(Bioneer, Cat. No. D-1040)
Lane 2: *Eco*RV digested DNA fragments
Lane 3: Ligation reaction with T4 DNA Ligase reaction buffer (E-3061) at 37 ℃ for 5 min
Lane 4: Ligation reaction with *AccuRapid*™ DNA Ligation Kit at 16} ℃ for 5 min
Lane 5: Ligation reaction with *AccuRapid*™ DNA Ligation Kit at 25 ℃ for 5 min
Lane 6: Ligation reaction with *AccuRapid*™ DNA Ligation Kit at 37 ℃ for 5 min

Ordering Information

Cat. No.	Product Description
K-7101	*AccuRapid*™ DNA Ligation Kit, 50 ligations
K-7102	*AccuRapid*™ DNA Ligation Kit, 150 ligations

03
DNA Ligase

Thermostable *Thermus filiformis* (*Tfi*) DNA Ligase

Description

Tfi DNA Ligase plays role of formatting phosphodiester bond by connecting double stranded DNA or connecting oligonucleotides 3'-hydroxyl end and 5'-phosphate end together.

Especially the reaction temperatures are between 45 and 65°C compared to different DNA Ligase such as T4 DNA Ligase, E. coli DNA Ligase, etc.

It is the higher temperature that keeps safe and active. In addition, *Tfi* DNA Ligase is possible to use in ligation reaction in high reaction temperature requirement.

Source

Tfi DNA Ligase is isolated from *E.coli* cells containing the ligase gene cloned from *Thermus filiformis*.

Applications

- Ligase Chain Reaction (LCR)
- Oligonucleotide Ligation Assay (OLA)
- Mutagenesis by Incorporation of a phosphorylated oligo during PCR Amplification
- Simultaneous Mutagenesis of Multiple Sites

Reagents supplied

- 10 x Reaction buffer (1 ml): 300 mM Tris-HCl (pH8.3), 250 mM KCl, 50 mM $MgCl_2$, 5 mM NAD
- 1 x Dilution buffer (1 ml): 10 mM Tris-HCl (pH7.6), 0.1 mM EDTA, 50 mM KCl, 1 mM DTT, 200 ug/mL acetylated BSA, 50% Glycerol

Storage Condition

20 mM Tris-HCl (pH7.6), 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.5% Tween -20, 0.5% IGEPAL CA-630, 50% Glycerol, store at -20℃

Concentration:

20 units/ul

Unit definition

One unit of *Tfi* DNA Ligase is defined as the amount of enzyme required to give 50% ligation of the 12 base pair cohesive ends of 1 ug of PspEI digested lambda DNA in 10min at 45℃.

Activity Assay Conditions

The activity assay is carried out in a 20 ul reaction containing 1 ug of PspEI digested lambda DNA and 1 x Tfi DNA ligase reaction buffer. After incubation at 45℃ for 10 min., the reaction is terminated by addition of stop solution (40%(w/v) sucrose, 50 mM EDTA and 0.25% bromophenol blue). Then heat at 70℃ for 10 min and immediately load on a 0.8% agarose gel.

Stability

The half-life of the enzyme in 1 x reaction buffer is more than 1 hour at 95℃ and 55 hours at 65℃.

Note: *Tfi* DNA Ligase should not be used as a substitute for other DNA ligase, i.e., T4 DNA Ligase.

References

- Barany, F. (1991) Proc. Natl. Acad. Sci. USA, 88, 189 - 193.
- Landegren, U. et al.(1988) Science 241, 1077 - 1080
- Michael, Scott F. (1994) Biotechniques 16:3, 410 - 412.
- Gerard J. A. et al. (1993) Biotechniques 15:1, 172 - 178.

Thermostable *Thermus filiformis* (*Tfi*) DNA Ligase

Experimental Data

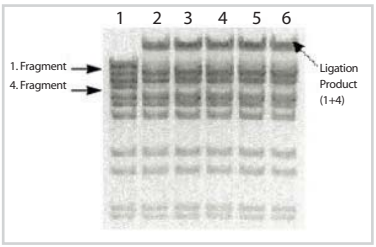


Figure 1. Ligation test at various temperatures (45℃ ~ 65℃)

Incubate the reaction containing ligase 1 unit and 1 ug DNA[lambda *PspEI*] at each temperature for 10 min.

Lane 1: λ DNA/PspE I (control)
Lane 2: Incubate at 45℃, 10 min
Lane 3: ncubate at 50℃, 10 min
Lane 4: Incubate at 55℃, 10 min
Lane 5: Incubate at 60℃, 10 min
Lane 6: Incubate at 65℃, 10 min

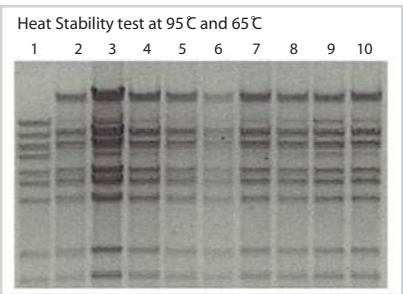


Figure 2. Heat Stability test at 95℃

Incubate the enzyme at 95℃ each time. And then add 1 unit ligase to a 20 ul reaction containing 1ug DNA[lambda *PspEI*] and incubate the mixture at 45℃ for 10 min.

Lane 1: {DNA/*PspEI*} (control)
Lane 2: Incubate at 95℃, 10 min
Lane 3: Incubate at 95℃, 20 min
Lane 4: Incubate at 95℃, 30 min
Lane 5: Incubate at 95℃, 40 min
Lane 6: Incubate at 95℃, 50 min
Lane 7: Incubate at 95℃, 60 min
Lane 8: Incubate at 95℃, 70 min
Lane 9: Incubate at 95℃ 80 min
Lane 9: Incubate at 95℃, 90 min

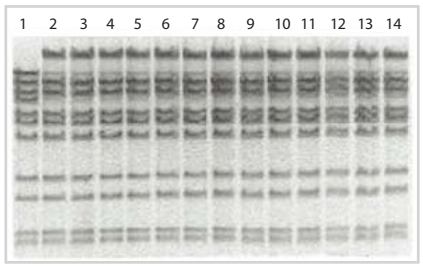


Figure 3. Heat Stability test at 95℃

Incubate the enzyme for each time at 65℃ and then add 1 unit ligase to a 20 ul reaction containing 1 ug DNA[lambda PspEI] and incubate the mixture at 45℃ for 10 min.

Lane 1: {DNA/*PspEI*} (control)
Lane 2: Incubate at 65°C, 6 hrs
Lane 3: Incubate at 65°C, 12 hrs
Lane 4: Incubate at 65°C, 18 hrs
Lane 5: Incubate at 65°C, 24 hrs
Lane 6: Incubate at 65°C, 30 hrs
Lane 7: Incubate at 65°C, 36 hrs
Lane 8: Incubate at 65°C, 42 hrs
Lane 9: Incubate at 65°C, 48 hrs
Lane 10: Incubate at 65°C, 54 hrs
Lane 11: Incubate at 65°C, 60 hrs
Lane 12: Incubate at 65°C, 66 hrs
Lane 13: Incubate at 65°C, 72 hrs
Lane 14: Incubate at 65°C, 78 hrs

Ordering Information

Cat. No.	Product Description
E-3111	*Tfi* DNA Ligase, 2,000 U, 10 x reaction buffer 1 ml, 1 x dilution buffer, 1 ml
E-3112	*Tfi* DNA Ligase, 10,000 U, 10 x reaction buffer 5 ml, 1 x dilution buffer, 5 ml

04 Restriction Enzyme

Restriction Enzyme

Restriction Enzymes

Restriction Enzymes

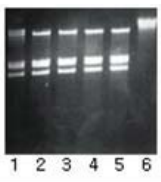
Bioneer provides 10x *AccuCut*™ buffer with every restriction endonuclease to exert optimal activity. This activity chart lists each restriction endonuclease and its optimal *AccuCut*™ buffer. It also gives the approximate activity of each restriction endonuclease. All *AccuCut*™ buffers are selected on the best conditions for multiple digest.

Unit determination
One unit of restriction endonuclease activity is defined the amount of enzyme required to completely digest 1 ug of substrate DNA in a total reaction volume of 50 ul in an hour using the *AccuCut*™ buffer provided. Incubations are performed in 1.5 ml tubes at the appropriate incubation temperature as indicated in the product profile.

Digestion Assay for Nuclease Contamination
All restriction endonuclease are incubated overnight in their recommended *AccuCut*™ buffer with 1 ug of substrate DNA in a volume of 50 ul. The characteristic banding pattern produced by the enzyme in one hour is compared to the pattern produced after overnight incubation. An unaltered pattern is an indication that the enzyme is free of detectable levels of nonspecific DNase. The maximum number of units that can be incubated overnight is given in product profile.

Assay for Exonuclease and Phosphatase Contamination
All restriction endonuclease are incubated with 0.1 ug of a 32P-labelled double strand oligonucleotide of 25-30 bp in 50 ul reaction volume with the supplied *AccuCut*™ buffer. Incubations are at the recommended temperature for 4 hours. Reaction products are separated by 20 % PAGE with 7M urea and are displayed by subsequent autoradiography of gel. Exonuclease and phosphatase contamination is determined as a percentage, calculated for 1 unit of the enzyme. All restriction endonucleases contain less than 0.05 % of the contamination activity.

Digestion-Ligation-Recutting
This assay is used to test for exonuclease activity that would degrade the termini of restriction fragments, resulting in inhibition of ligation and of subsequent digestion of ligated fragments. DNA fragments are produced by an excessive over-digestion of substrate DNA with each restriction endonuclease. The fragments obtained and then ligated. The ligated fragments are then recut with the same restriction endonuclease. A normal banding pattern after cleavage indicates that both the 3' and 5' termini are intact and the enzymes preparation is free of detectable exonuclease and phosphatase.

Results for digestion	Overdigestion assay	Ligation and recutting assay
		
Lane 1 : 1 U of *Bam*H I Lane 2 : 1.5 U of *Bam*H I Lane 3 : 2 U of *Bam*H I Lane 4 : 3 U of *Bam*H I Lane 5 : 10 U of *Bam*H I Lane 6 : Lambda DNA	Lane 1 : Lambda DNA Lane 2 : 60 U of *Bam*H I Lane 3 : 120 U of *Bam*H I	Lane 1 : Cut Lane 2 : Ligate Lane 3 : Recut

04 Restriction Enzyme

Restriction Enzymes

Blue-White Selection Assay

Enzymes used for cloning application are tested by an additional quality control, the Blue-White Screening Assay, to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. The assay is performed by digesting an appropriate vector at a unique site with a 5-fold excess of enzyme, ligating, transforming into JM109 cells and plating on X-gal/ ITPG/ Amp plates. The percentage of white colonies versus blue colonies is deter mind. Enzymes that generate overhangs must produce fewer than 2% white colonies and blunt cutting enzymes must produce fewer than 5% white colonies. All restriction enzymes tested must produce fewer than 3% white colonies in order to be Blue-White certified.

10X Reaction Buffer

Bioneer provides a color coated 10X reaction buffer with each of the restriction endonuclease to ensure optimal activity. The buffer should be used at 10 times-diluted concentration in the reaction.

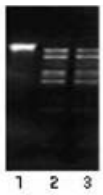
Storage

All the enzymes must be stored at -20 °C.

Buffer systems

- *AccuCut*™ buffer Blue (B) 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 8.5
- *AccuCut*™ buffer violet (I) 33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM potassium-acetate, 1 mM DTT, pH 7.9
- *AccuCut*™ buffer Orange (O) 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6
- *AccuCut*™ buffer greeN (N) 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6
- *AccuCut*™ buffer Red (R) 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6

* Concentration of each enzymes is dependent on the batches of production. To know exact concentration of enzymes, please see the insert sheet in the each enzyme package.

Results for digestion	Overdigestion assay	Ligation and recutting assay
		
Lane 1 : Lambda DNA Lane 2 : 1 U of *Ssp* I Lane 3 : 2 U of *Ssp* I Lane 4 : 3 U of *Ssp* I Lane 5 : 10 U of *Ssp* I	Lane 1 : Lambda DNA Lane 2 : 10 U of *Ssp* I Lane 3 : 20 U of *Ssp* I	Lane 1 : Cut Lane 2 : Ligate Lane 3 : Recut

Restriction Enzymes

Restriction Endonuclease Activity in the Five Standard *AccuCut*™ buffer

Enzyme	Optimal Buffer	Activity in AccuCut™ buffers (%)					Optimal Temp ℃
		B	I	O	N	R	
Spe I(*Acl*N I)	N	25-50	75-100	75-100	100	10-25	37
Afe I	I	75-100	100	25-50	0-25	75-100	37
Alu I	N	50-75	75-100	75-100	100	0-25	37
Apa I	I	0-10	100	25-50	50-75	0-10	37
Hha I(*Asp*LE I)	R	50-75	25-50	75-100	0-25	100	37
Nhe I(*Asu*NH I)	N	0-10	75-100	50-75	100	0-10	37
*Bam*H I	O	75-100	25-50	100	25-50	75-100	37
Bgl II	R	25-50	10-25	10-25	0-10	100	37
Cla I(*Bsa*29 I)	O	50-75	75-100	100	25-50	50-75	37
Nco I(*Bsp*19 I)	B	100	10-25	25-50	0-10	75-100	37
*Bst*N I(*Bst*2U I)	O	50-75	10-25	100	75-100	50-75	60
Dde I(*Bst*DE I)	O	50-75	10-25	100	75-100	25-50	60
Not I(*Cci*N I)	I	75-100	100	50-75	25-50	75-100	37
Dra I	O	75-100	75-100	100	75-100	25-50	37
*Eco*R I	*Eco*R I	100	50-75	75-100	50-75	75-100	37
*Eco*R V	B	100	25-50	25-50	0-10	50-75	37
Fau I	N	0-10	50-75	25-50	100	0-10	55
Nde I(*Fau*ND I)	I	50-75	100	75-100	50-75	10-25	37
Hae III	O	50-75	50-75	100	75-100	25-50	37
Hind III	B	100	0-10	25-50	10-25	0-10	37
Hinf I	R	75-100	75-100	75-100	25-50	100	37
Kpn I	N	25-50	75-100	25-50	100	25-50	37
Dpn II(*Kzo*9 I)	O	50-75	50-75	100	50-75	50-75	37
Mlu I	R	25-55	10-25	10-25	0-10	100	37
Nar I(*Mly*113 I)	N	10-25	50-75	25-50	100	10-25	37
Msp I	N	75-100	75-100	75-100	100	50-75	37
Sac I(*Psp*124B I)	O	0-10	75-100	100	75-100	10-25	37
Pst I	R	25-50	25-50	25-50	10-25	100	37
Pvu II	O	25-50	25-50	100	25-50	25-50	37
Rsa I	N	50-75	75-100	50-75	100	0-10	37
Sal I	R	25-50	0-10	10-25	0-10	100	37
*Sfa*N I	R	75-100	0-10	25-50	10-25	100	37
Xho I(*Sfr*274 I)	N	50-75	75-100	75-100	100	50-75	50
Sma I	I	0-10	100	0-10	0-10	0-10	25
Sph I (*Bbu* I)	O	75-100	50-75	100	25-50	75-100	37
Mse I(*Tru*9 I)	B	100	50-75	25-50	75-100	25-50	65
Ase I(*Vsp* I)	B	100	25-50	10-25	0-10	50-75	37
Xba I	R	50-75	75-100	75-100	75-100	100	37
Xma I	I	0-10	100	50-75	75-100	0	37

04 Restriction Enzyme

Restriction Enzymes

Restriction Enzymes Isoschizomer Contents

Bioneer Enzyme	Isoschizomer	Site
*Acc*16 I	*Aos* I, *Avi* II, *Fsp* I, *Mst* I, *Nsb* I	TGC▾GCA
*Acc*65 I	*Asp*718 I, *Sth* I	G▾GTACC
*Acc*113 I	*Dpa* I, *Eco*255 I, *Sca* I	AGT▾ACT
*Acc*B1 I	*Ban* I, *Hgic* I, *Bbv*B I, *Eco*64 I, *Msp*B4 I	G▾G(C/T)(G/A)CC
*Acc*B7 I	*Acp* II, *Asp*10H II, *Esp*1396 I, *Van*91 I, *Pfl*M I	CCANNNN▾NTGG
*Acc*BS I	*Bsr*B I, *Mbi* I	GAG▾CGG
Acl I	*Psp*1406 I	AA▾CGTT
*Acl*N I	*Spe* I	A▾CTAGT
*Acl*W I	*Bin* I, *Alw* I, *Bsp*P I	GGATCNNNN▾
Acs I	*Apo* I, *Fsi* I, *Xap* I	(G/A)▾AATT(C/T)
Afe I	*Ait* I, *Aor*51H I, *Fun* I, *Eco*47 III	AGC▾GCT
Alu I	*Mlt* I	AG▾CT
*Ama*87 I	*Aqu* I, *Ava* I, *Bco* I, *Bso*B I, *Eco*27K I, *Eco*88 I, *Nsp*II, *Nsp*SA I	C▾(C/T)CG(G/A)G
Apa I	*Ppe* I	GGGCC▾C
*Asi*A I	*Age* I, *Pin*A I, *Bsh*T I	A▾CCGGT
*Asp*LE I	*Cfo* I, *Fnu*D III, *Hha* I	GCG▾C
*Asp*S9 I	*Asu* I, *Avc* I, *Bsp*B II, *Bsu*54 I, *Cfr*13 I, *Sau*96 I	G▾GNCC
*Asu*C2 I	*Aha* I, *Bcn* I, *Cau* II, *Hgi*S22 I, *Nci* I	CC▾(C/G)GG
*Asu*HP I	*Hph* I	GGTGANNNNNNNN▾
*Asu*NH I	*Nhe* I, *Pst*NH I	G▾CTAGC
*Bam*H I	*Acc*EB I, *Ali* I, *Bna* I, *Bst* I, *Nsp*SA IV, *Sur* I	G▾GATCC
*Bbv*12 I	*Alw*21 I, *Asp*H I, *Bsh*45 I, *Hgi*A I, *Bsi*HKA I	G(A/T)GC(A/T)▾C
Bgl I	*Tsp*8E I	GCCNNNN▾NGGC
Bgl II	*Ncr* I, *Nsp*MAC I, *Pae*2k I, *Pae*18k I	A▾GATCT
*Bme*18 I	*Afl* I, *Ava* II, *Bme*216 I, *Cau* I, *Eco*47 I, *Hgi*B I, *Hgi*E I, *Sin* I	G▾G(A/T)CC
*Bpu*14 I	*Acp* I, *Asp*10H I, *Asu* II, *Bsp*119 I, *Bst*B I, *Csp*45 I, *Fsp* II, *Lsp* I, *Nsp* V, *Sfu* I, *Ssp*1 I	TT▾CGAA
*Bsa*29 I	*Aag* I, *Ban* III, *Bci*29 I, *Bsc* I, *Bsp*106 I, *Bsu*15 I, *Cla* I	AT▾CGAT
*Bsc*4 I	*Bsi*Y I, *Bsl* I, *Bsa*L I	CCNNNNN▾NNGG
*Bse*1 I	*Bse*N I, *Bsr* I, *Bsr*S I, *Bst*11 I, *Tsp*1 I	ACTG▾G
*Bse*3D I	*Bsr*D I, *Bse*M I	GCAATGNN▾
*Bse*8 I	*Bsa*B I, *Bsh*1365 I, *Bsi*B I, *Bsr*BR I, *Mam* I	GATNN▾NNATC
*Bse*21 I	*Aoc* I, *Axy* I, *Bst*29 I, *Bst*30 I, *Bsu*36 I, *Cvn* I, *Eco*81 I, *Mst* II, *Sau* I, *Ssh*A I	CC▾TNAGG
*Bse*118 I	*Bco*118, *Bsr*F I, *Bss*A I, *Cfr*10 I	(G/A)▾CCGG(C/T)
*Bse*P I	*Bss*H II, *Pau* I	G▾CGCGC
*Bse*X3 I	*Aaa* I, *Bst*Z I, *Eag* I, *Ecl*X I, *Eco*52 I, *Xma* III	C▾GGCCG
*Bsp*13 I	*Acc* III, *Bse*A I, *Bsi*M I, *Bsp*E I, *Bsp*M II, *Bsu*23 I, *Kpn*2 I, *Mro* I, *Pta* I	T▾CCGGA
*Bsp*19 I	*Nco* I	C▾CATGG
*Bsp*1720 I	*Blp* I, *Bpu*1102 I, *Cel* II, *Esp* I	GC▾TNAGC
*Bsp*A2 I	*Avr* II, *Avr*B II, *Bln* I	C▾CTAGG
*Bss*NA I	*Bsp*M90 I, *Bst*BS I, *Bst*1107 I, *Sna* I, *Xca* I	GTA▾TAC
*Bss*T1 I	*Eco*T14 I, *Eco*130 I, *Erh* I, *Erh*B9 II, *Sty* I	C▾C(A/T)(A/T)GG
*Bst*2B I	*Bsi* I, *Bss*SI	C▾TCGTG

Restriction Enzymes

*Bst*2U I	*Aor* I, *Apy* I, *Bse*B I, *Bse*16 I, *Bse*17 I, *Bse*24 I, *Bsp*N I, *Bst*2 I, *Bst*N I, *Bst*O I, *Eco*R II, *Fsp*1604 I, *Mva* I, *Sth*117 I, *Zan* I	CC▾(A/T)GG
*Bst*AC I	*Acy* I, *Aha* I, *Asu* III, *Bbi* II, *Bsa*H I, *Hgi* I, *Hin*1 I, *Msp*17 I, *Pam* II	G(G/A)▾CG(C/T)C
*Bst*AP I	*Apa*B I	GCANNNN▾NTGC
*Bst*BA I	*Bsa*A I, *Msp*Y I	(C/T)AC▾GT(G/A)
*Bst*DE I	*Dde* I	C▾TNAG
*Bst*DS I	*Dsa* I	C▾C(A/G)(C/T)GG
*Bst*F5 I	*Bse*G I	GGATGNN▾
*Bst*H2 I	*Acc*B2 I, *Bsp*143 II, *Hae* II	(G/A)GCGC▾(C/T)
*Bst*HP I	*Hpa* I	GTT▾AAC
*Bst*MC I	*Bsa*O I, *Bsh*1285 I, *Bsi*E I, *Mcr* I	CG(G/A)(C/T)▾CG
*Bst*NS I	*Nsp* I, *Nsp*H I	(G/A)CATG▾(C/T)
*Bst*SF I	*Bdi*S I, *Lla*B I, *Sfc* I, *Sfe* I	C▾T(G/A)(C/T)AG
*Bst*SN I	*Eco*105 I, *Sna* B I	TAC▾GTA
*Bst*X2 I	*Bst*Y I, *Mfl* I, *Tru*201 I, *Xho* II	(G/A)▾GATC(C/T)
*Bsu*6 I	*Bco*5 I, *Bco*116 I, *Bse*Z I, *Ear* I, *Ksp*632 I	CTCTTCN▾
*Bsu*R I	*Bim*19 II, *Bsh* I *Bsp*K I, *Bsp*R I, *Bsp*211 I, *Dsa* II, *Fnu*D I, *Hae* III, *Pla* I, *Sbv* I, *Sfa* I	GG▾CC
*Cci*N I	*Not* I	GC▾GGCCGC
Dra I	*Aha* III	TTT▾AAA
*Dse*D I	*Drd* I	GACNNNN▾NNGTC
*Eco*R I	*Hal* I, *Kpn*49k I, *Rsr* I, *Sso* I	G▾AATTC
*Eco*R V	*Ceq* I, *Eco*32 I	GAT▾ATC
Ege I	*Ehe* I, *Eco*78 I	GGC▾GCC
Erh I	*Bss*T1 I, *Eco*T14 I, *Eco*130 I, *Erh*B9 II, *Sty* I	C▾C(A/T)(A/T)GG
Fau I	None	CCCGCNNNN▾
*Fau*ND I	*Nde* I	CA▾TATG
Fok I	None	GGATG(9)▾
*Fri*O I	*Ban* II, *Bsu*1854 I, *Bsp*519 I, *Bvu* I, *Eco*24 I, *Eco*215 I, *Hgi*J II, *Sac*N I	G(G/A)GC(C/T)▾C
*Fsp*4H I	*Bso*F I, *Bsp*6 I, *Fbr* I, *Fnu*4H I, *Ita* I, *Uur*960 I	GC▾NGC
Hae III	*Bim*19 II, *Bsh* I, *Bsp*K I, *Bsp*211 I, *Bsu*R I, *Dsa* II, *Fnu*D I, *Pla* I, *Sbv* I, *Sfa* I	GG▾CC
Hind III	*Bst*F I, *Eco*V III, *Hsu* I, *Ssb* I	A▾AGCTT
*Hin*f I	*Cvi*B I, *Fnu*A I, *Hha* II	G▾ANTC
Hpa II	*Bco*27 I, *Bsi*S I, *Bst*40 I, *Hap* II, *Msp* I, *Sth*134 I	C▾CGG
*Hsp*A I	*Hin*P1 I, *Hin*6 I, *Sci*N I	G▾CGC
Kpn I	None	GGTAC▾C
*Ksp*22 I	*Atu*C I, *Bco*102 I, *Bsp*X II, *Fba* I, *Pov* I	T▾GATCA
*Kzo*9 I	*Asp*MD I, *Bsp*A I, *Bsp*105 I, *Bsp*143 I, *Btk* II, *Dpn* II, *Mbo* I, *Nde* II, *Nla* II, *Sau*3A I	▾GATC
Mlu I	None	A▾CGCGT
*Mly*113 I	*Mch* I, *Nar* I, *Nda* I, *Nun* II, *Sse*A I	GG▾CGCC
*Mro*N I	*Eco*56 I, *Ngo*A IV, *Ngo*M I	G▾CCGGC
*Mro*X I	*Asp*700 I, *Bbu*A I, *Xmn* I	GAANN▾NNTTC
Msp I	same as *Hpa* II	C▾CGG
*Msp*R9 I	*Bme*1390 I, *Msp*67 I, *Scr*F I	CC▾NGG

04 Restriction Enzyme

Restriction Enzymes

Nru I	*Bsp*68 I, *Mlu*B2 I, *Sbo*13 I, *Spo* I	TCG▾CGA
*Nru*G I	*Ahd* I, *Asp*E I, *Eam*1105 I, *Ecl*HK I, *Uba*1190 I, *Uba*1191 I	GACNNN▾NNGTC
*Ple*19 I	*Bsp*C I, *Erh*B9 I, *Nb*1 I, *Ple*19 I, *Pvu* I, *Rsh* I, *Xor* II	CGAT▾CG
*Pme*55 I	*Aat* I, *Eco*147 I, *Sse*B I, *Stu* I	AGG▾CCT
*Psp*124B I	*Sac* I, *Sst* I	GAGCT▾C
*Psp*E I	*Acr* II, *Asp*A I, *Bst*E II, *Bst*P I, *Eca* I, *Eco*O65 I, *Eco*91 I, *Nsp*SA II	G▾GTNACC
*Psp*L I	*Bpu*B5 I, *Bsi*W I, *Pfl*23 II, *Ppu*A I, *Sp*1 I, *Sun* I	C▾GTACG
*Psp*N4 I	*Asp*N I, *Bsc*B I, *Nla* IV	GGN▾NCC
*Psp*OM I	*Bsp*120 I	G▾GGCCC
*Psp*PP I	*Pfl*27 I, *Ppu*M I, *Psp*5 II	(G /A)▾GG(A/T)CC(C/T)
Pst I	*Api* I, *Asp*713 I, *Bsp*B I, *Bsp*63 I, *Hal* II, *Sfl* I	CTGCA▾G
Pvu II	*Bav* I, *Dma* I, *Pvu*84 II	CAG▾CTG
Rsa I	*Afa* I	GT▾AC
Sal I	*Hgi*C III, *Hgi*D II, *Nop* I, *Xci* I	G▾TCGAC
Sbf I	*Sse*8387 I	CCTGCA▾GG
*Sfa*N I	None	GCATCNNNNN▾
Sfi I	*Sdi* I	GGCCNNNN▾NGGCC
*Sfr*274 I	*Abr* I, *Blu* I, *Ccr* I, *Mav* I, *Pae*R7 I, *Pan* I, *Sla* I, *Xho* I, *Xpa* I	C▾TCGAG
*Sfr*303 I	*Cfr*42 I, *Csc* I, *Gal* I, *Kpn*19 I, *Ksp* I, *Sac* II, *Sst* II	CCGC▾GG
Sma I	*Cfr*J4 I, *Pae*B I, *Psp*AL I	CCC▾GGG
Smi I	*Swa* I	ATTT▾AAAT
Sph I	*Bbu* I, *Pae* I	GCATG▾C
*Sse*9 I	*Tsp*E I, *Tsp*509 I	▾AATT
Ssp I	unfound	AAT▾ATT
*Tru*9 I	*Mse* I, *Tru*1 I	T▾TAA
*Tth*111 I	*Asp* I, *Ats* I	GACN▾NNGTC
*Vha*464 I	*Afl* II, *Bfr* I, *Bsp*T I, *Bst*98 I, *Esp*4 I, *Msp*C I	C▾TTAAG
Vne I	*Aaq* I, *Alw*44 I, *Apa*L I, *Sno* I	G▾TGCAC
Vsp I	*Ase* I, Asn I, PshB I, Vsp I	AT▾TAAT
Xba I	None	T▾CTAGA
Xma I	*Ahy* I, *Cfr*9 I, *Eae*A I, *Psp*A I, *Xcy* I, *Xma*C I	C▾CCGGG
*Zsp*2 I	*Eco*T22 I, *Mph*1103 I, *Nsi* I, *Pin*B I, *Sep* I	ATGCA▾T

Restriction Enzymes

Panlindromic Teta-and Hexa-Nucleotide Recognition Sequences

	AATT	ACGT	AGCT	ATAT	CATG	CCGG	CGCG	CTAG	GATC	GCGC	GGCC	GTAC	TATA	TCGA	TGCA	TTAA
↓□□□□	*Ssep* I *Tsp*E I							*Kzo*9 I *Bsp*143 I *Mbo* I								
□↓□□□		*Mae* II				*Hpa* II *Msp* I *Hin*2 I		*Mae* I		*Hsp* I *Hin*6 I		*Csp*6 I		*Taq* I		*Tru*9 I *Tru*1 I
□□↓□□			*Alu* I				*Bsh*1236 I		*Dpn* I		*Bsu*R I *Hae* III	*Rsa* I				
□□□↓□										*Asp*LE I *Hha* I						
□□□□↓		*Tai* I			*Nla* II											
A↓□□□□T	*Apo* I		*Hind* III		*Bsp*LU11 I	*Asi*A I *Age* I *Bsa*W I *Cfr*10	*Mlu* I *Afl* I	*Acl*N I *Spe* I	*Bgi* I *Xho* II						*Pru*10 I	
A□↓□□□T		*Acl* I *Rsp*1406 I												*Bsa*29 I *Bsu*15 I		*Vap* I
A□□↓□□T				*Sap* I						*Afe* I *Eco*47 I	*Pme*55 I *Eco*147 I	*Acc*13 I *Eco*255 I *Sca* I				
A□□□↓□T																
A□□□□↓T					*Nsp* I					*Bsp*142 II					*Zsp*2 I *Mph*103 I	
C↓□□□□G					*Bsp*19 I *Bse*D I *Bsa* I *Eco*130 I *Nco* I	*Xma* I *Bse*D I *Cfr*9 I *Eco*88 I	*Bse*D I *Dsa* I	*Bsp*A2X I *Avr* I *Bse*D I *Eco*130 I			*Bse*X3 I *Cfr* I *Eoc*52 I	*Psp*L I *Pff*23 II	*Bfm* I	*Sfr*274 I *Eco*88 I *Xho* I	*Bfm* I	*Vha*464 I *Bsp*T I
C□↓□□□G				*Fau*ND I *Nde* I												
C□□↓□□G		*Eco*72 I *Bsa*A I	*Pvu* II *NapB* II			*Sme* I	*Nsp*B II									
C□□□↓□G							*Sfr*303 I *Cfr*42 I		*Ple*19 I *Bsh*1258 I *Pvu* I	*Bsh*1258 I						
C□□□□↓G															*Pst* I	
G↓□□□□C	*Eoc*R I *Api* I					*Nio*n I *Cfr*42 I *Ngo*M I	*Bse*P I *Pau* I	*Asu*NH I *Nhe* I	*Bam*H I *Xho* II	*Bsh*N I *Eco*64 I *Kas* I	*Psp*CM I *Bsp*120 I	*Acc*65 I *Bsh*N I *Eco*64 I		*Sal* I	*Vne* I *Alw*44 I	
G□↓□□□C		*Hin*1 I								*Mly*113 I *Hin*1 I *Nar* I			*Acc* I	*Acc* I		
G□□↓□□C			*Ecl*136 II	*Eco*RV *Eco*32 I	*Cac*8 I	*Nae* I *Cae*8 I	*Cac*8 I	*Cac*8 I	*Bsp*L I	*Ege* I *Ehe* I *Bsp*L I	*Bsp*L I	*Bsp*L I	*Bss*NA I *Bst*1107 I	*Hinc* II		*Bst*HP I *Hinc* II *Hpa* I
G□□□↓□C																
G□□□□↓C		*Aat* II	*Psp*LE I *Alw*21 I *co*24 I *Sac* I *Sdu* I		*Sph* I *Pae* I *Nsp* I					*Bsp*143 II *Bbe* I	*Apa* I *Eco*24 I *Sdu* I	*Kpn* I			*Alw*21 I *Sdu* I	
T↑□□□□A					*Bsp*H I	*Bsp*13 I *Bsa*W I *Kpn*2 I		*Xba* I	*Bcl* I		*Cfr* I	*Ksp*22 I *Bsp*1407 I				
T□↑□□□A															*Bpu*14 I *Bsp*119 I	
T□□↑□□A		*Bst*SN I *Eco*105 I *Bsa*A I					*Nru* I *Bsp*68 I			*Acc*16 I *Fsp* I	*Bal* I					*Dra* I
T□□□↑□A																
T□□□□↑A																

04 Restriction Enzyme

Restriction Enzymes

Restriction Enzymes

Afe I	▼ 5'…AGCGCT…3' 3'…TCGCGA…5' ▲	unit 200 1,000	Cat. No. E-1111 E-1112

Source: *Alcaligenes faecalis* T2774.
■ **Reaction Condition**: *AccuCut*™ buffer vlolet :
33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate, 1 mM DTT, pH 7.9, **Incubate at 37℃ .**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 10,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)

Isoschizomer: *Ait* I, *Aor*51H I, *Fun* I, *Eco*47 III.
Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Unidentified

Alu I	▼ 5'…AGCT…3' 3'…TCGA…5' ▲	unit 500 2,500	Cat. No. E-1121 E-1122

Source : *Arthrobacter luteus*.
■ **Reaction Condition**: *AccuCut*™ buffer greeN :
10 mM Tris-HCL, 10 mM $MgCl_2$, 1 mM DTT, pH 7-6, **Incubate at 37℃.**
Storage Buffer: 110 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 10,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)

Isoschizomer: *Bsa*L I, *Mar* I, *Mlt* I, *Otu* I.
Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Unidentified

Apa I	▼ 5'…GGGCCC…3' 3'…CCCGGG…5' ▲	unit 2,500 12,500	Cat. No. E-1141 E-1142

Source : *Acetobacter Pasteurianus*.
■ **Reaction Condition**: *AccuCut*™ buffer vlolet :
33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate, 1 mM DTT, pH 7.9, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 20,000-100,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Ppe* I.

▼
Neoschizomer: *Psp*OM I (GGGCCC).
Reactivity on methylated substrate
DNA : Blocked by over lapping dcm methylation ($C^{m5}CWGG$), $GGGCCm^5C$.

*Asp*LE I	▼ 5'…GCGC…3' 3'…CGCG…5' ▲	unit 1,000 5,000	Cat. No. E-1161 E-1162

Source : *Acetobacter species LE 386*.
■ **Reaction Condition**: *AccuCut*™ buffer Red :
50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2- mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: 10,000 units/mL

Heat Inactivation: No
Isoschizomer: *Cfo* I, *Fnu* D III, *Hha* I.
Neoschizomer: Hin P I(GCGC), ▼Hsp A I(GCGC)▼
Reactivity on methylated substrate
DNA : anidentified

Restriction Enzymes

Vsp I (*Ase* I)	▼ 5'…ATTAAT…3' 3'…TAATTA…5' ▲	unit 1,000 5,000	Cat. No. E-2141 E-2142

Source : *Vibrio species* 343.
■ **Reaction Condition**: *AccuCut*™ buffer Blue :
10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 8.5,
Incubate at 37℃.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 10,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Ase* I, *Asn* I, *PshB* I, *Vsp* I.
Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Unidentified

*Bam*H I	▼ 5'…GGATCC…3' 3'…CCTAGG…5' ▲	unit 5,000 25,000	Cat. No. E-1211 E-1212

Source : *Bacillus amyloliquefaciens* H.
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl,
1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer : 10 mM Tris-HCl, 50 mM KCl, 0.1 mM
EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5,
Store at -20℃.
Concentration: * 50,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Acc*EB I, *Ali* , *Bna* I, *Bst* I, *Nsp*SA IV, *Sur* I

Neoschizomer: Unfound
Reactivity on methylated substrate
DNA : Not blocked by overlapping dam methylation
G^{m5}ATC, GGATC^{m5}C, GG^{m6}ATCC,
GG ^{m6}ATC^{m5}, GGATC^{m4}C :
Blocked by GGA T^{m4}CC#, GGA t^{m5}CC,
GGATh^{m5}C^{m5}C, GGAh^{m5}UCC.

Sph I (*Bbu* I)	▼ 5'…GCATGC…3' 3'…CGTACG…5' ▲	unit 200 1,000	Cat. No. E-2071 E-2072

Source : *Streptomyces phaeochromogenes.*
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl,
1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃.
Concentration: * 5,000 units/ml.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Bbu* I, *Pae* I

Neoschizomer: Unfound
Reactivity on methylated substrate
DNA : Blocked by GC^{m6}ATGC, Gh^{m5}CATGh^{m5}C.
Not blocked by GCATG^{m5}C.

Restriction Enzymes

		unit	Cat. No.
Bgl II	▼ 5'…AGATCT…3' 3'…TCTAGA…5' ▲	1,000 5,000	E-1241 E-1242

Source: *Bacillus globigii*.
■ **Reaction Condition**: *AccuCut*™ buffer Red :
50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2- mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 20,000 units/mL.

Heat Inactivation: No.
Isoschizomer: Ncr I, NspMAC I, Pae2k I, Pae18k I.
Neoschizomer: Unfound
Reactivity on methylated substrate
DNA : Not blocked by GC^{m5}CN5GGCb:
Blocked by G^{m5}CCN5GGC, GCCN5GG^{m5}C GC ^{m4}CN5GGC

		unit	Cat. No.
*Bst*2U I (*Bst*N I)	▼ 5'…CC(A/T)GG…3' 3'…GG(T/A)CC…5' ▲	2,000 10,000	E-1431 E-1432

Source: *Bacillus stearothermophilus 2U*.
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 60℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 20,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Aor* I, *Apy* I, *Bse*B I, *Bse*16 I, *Bse*17 I, *Bse*24 I, *Bsp*N I, *Bst*2 I, *Bst*N I, *Bst*O I, *Eco*R II, *Fsp*1604 I, *Mva* I, *Sth*117 I, *Zan* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Not blocked by C^{m5}CWGG.

		unit	Cat. No.
*Bsa*29 I(*Cla* I)	▼ 5'…ATCGAT…3' 3'…TAGCTA…5' ▲	600 3,000	E-1271 E-1272

Source: *Bacillus stearothermophilus* 29.
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 15,000 units/mL.

Heat Inactivation : Yes. (65℃ for 20 minutes)
Isoschizomer: *Aag* I, *Ban* III, *Bci*29 I, *Bsc* I, *Bsp*106 I, *Bsu*15 I, *Cla* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by G^{m5}ATC.

Restriction Enzymes

*Bst*DE I (*Dde* I)	▼ 5'…CTNAG…3' 3'…GANTC…5' ▲	unit 400 2,000	Cat. No. E-1471 E-1472

Source: *Bacillus stearothermophilus* DE.
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 60℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 20,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Dde* I.
Neoschizomer: Unfound

Reactivity on methylated substrate
DNA: Unidentified

*Kzo*9 I(*Dpn* II)	▼ 5'…GATC…3' 3'…CTAG…5' ▲	unit 200 1,000	Cat. No. E-1781 E-1782

Source : *Kurthia zopfil* 9.
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 5,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Asp*MD I, *Bsp*A I, *Bsp*105 I, *Bsp*143 I, *BtK* II, *Dpn* II, *Mbo* I, *Nde* II, *Nla* II, *Sau*3A I

Neoschizomer: *Dpn* I (GATC).

Reactivity on methylated substrate
DNA : Not blocked by $G^{m5}ATC$.

Dra I	▼ 5'…TTTAAA…3' 3'…AAATTT…5' ▲	unit 2,000 10,000	Cat. No. E-1601 E-1602

Source: *Deinococcus radiophilus.*
■ **Reaction Condition**: *AccuCut*™ buffer Orange:
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 10,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Aha* III.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by $TTTA^{m6}AA$

Restriction Enzymes

*Eco*R I	▼ 5'…GAATTC…3' 3'…CTTAAG…5' ▲	unit 5,000 25,000	Cat. No. E-1621 E-1622

Source: *Escherichia coli.*
Reaction Condition: *AccuCut*™ buffer *Eco*R1:
100 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *20,000-100,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Hal* I, *Kpn*49k I, *Rsr* I, *Sso* I.
Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by $G^{m6}ATTC$, $GA\ ^{m6}ATTC$, $GAAT\ T^{m5}C$
Not blocked by $GAATTh^{m5}C$, $GAAh^{m5}Uh^{m5}UC$.

*Eco*R V	▼ 5'…GATATC…3' 3'…CTATAG…5' ▲	unit 2,000 10,000	Cat. No. E-1631 E-1632

Source: *Escherichia coli.*
■ **Reaction Condition**: *AccuCut*™ buffer Blue :
10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 8.5, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 20,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Ceq* I, *Eco*32 I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by $G^{m6}ATATC$, $GA\ T^{m6}ATC$
Not blocked by $GATA\ T^{m5}C$, $GATATh^{m5}C$

Fau I	▼ 5'…CCCGCNNNN…3' 3'…GGGCGNNNNNN…5'a ▲	unit 50 250	Cat. No. E-1661 E-1662

Source: *Flavobacterium aquatili.*
■ **Reaction Condition**: *AccuCut*™ buffer greeN :
10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6, **Incubate at 55℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 500 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *None*

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Unidentified

Hae III	▼ 5'…GGCC…3' 3'…CCGG…5' ▲	unit 3,000 15,000	Cat. No. E-1711 E-1712

Source : *Haemophilus aegyptius.*
■ **Reaction Condition**: *AccuCut*™ buffer Orange :
10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: 10,000 units/mL

Heat Inactivation: No.
Isoschizomer: *Bin*19 II, *Bsh* I, *Das* II, *Sfa* I.

Neoschizomer: Un found

Reactivity on methylated substrate
DNA : Unidentified

Restriction Enzymes

		unit	Cat. No.
Hind III	▼ 5'…AAGCTT. …3' 3'…TTCGAA…5' ▲	10,000 50,000	E-1721 E-1722

Source : *Haemophilus influenzae Rd.*
■ **Reaction Condition**: *AccuCut*™ buffer Blue : 10 mM Tris-HCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 8.5, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration : *20,000-100,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *BstF* I, *Eco*V III, *Hsu I*, *Ssb* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by AAGh^{m5}CTT, AAG^{m5}CTT, ^{m6}AAGCTT. Not blocked by AAGCh^{m5}Uh^{m5}U, A ^{m6}AGCTT.

		unit	Cat. No.
Hinf I	▼ 5'…GANTC…3' 3'…CTNAG…5' ▲	2,000 10,000	E-1731 E-1732

Source : *Haemophilus influenzae.*
■ **Reaction Condition**: *AccuCut*™ buffer Red : 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *20,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *CviB* I, *FnuA* I, *Hha* II.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by G^{m6}ANTC, GANTh^{m5}C. Not blocked by GANT ^{m5}C.

		unit	Cat. No.
Hpa II	▼ 5'…CCGG…3' 3'…GGCC…5' ▲	1,000 5,000	E-1741 E-1742

Source: Haemephilns parainfluenzae.
■ **Reaction Condition**: *AccuCut*™ buffer greeN : 10mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 10,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Msp* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by ^{m4}CCGG, ^{m5}CCGG, C^{m4}CGG. C^{m5}CGG, ^{m5}C^{m5}CGG.

		unit	Cat. No.
Msp I	▼ 5'…CCGG…3' 3'…GGCC…5' ▲	3,000 15,000	E-1831 E-1832

Source : *Moraxella species.*
■ **Reaction Condition**: *AccuCut*™ buffer greeN : 10mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: 20,000 units/mL

Heat Inactivation: Yes(65℃ for 20 minutes).
Isoschizomer: *Hpa* II.

Neoschizomer: Un found

Reactivity on methylated substrate
DNA : Blocked by ^{m5}CCGG, ^{m5}C^{m5}CGG
Not blocked by ^{m4}CCGG, C^{m4}CGG, C^{m5}CGG

04 Restriction Enzyme

Restriction Enzymes

Kpn I	5'…GGTACC…3' 3'…CCATGG…5'	unit 3,000 15,000	Cat. No. E-1761 E-1762

Source : *Klebsiella pneumonia*
■ **Reaction Condition**: *AccuCut*™ buffer greeN : 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *15,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Acc*65 I, *Asp*718, *Asp*718 I (GGTACC)

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by GG T^{m6}ACC, GGTA^{m4}CC, GGTA^{m5}C^{m5}C, GGTAC^{m4}C. Not blocked by GGTA^{m5}CC, GGTAC^{m5}C

Mlu I	5'…ACGCGT…3' 3'…TGCGCA…5'	unit 1,000 5,000	Cat. No. E-1791 E-1792

Source : *Micrococcus luteus.*
■ **Reaction Condition** : *AccuCut*™ buffer Red : 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer : 10mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *20,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer : *None.*

Neoschizomer : Unfound

Reactivity on methylated substrate
DNA : Blocked by A^{m5}CGCGT. Not blocked by ^{m6}ACGCGT.

*Tru*9 I (*Mse* I)	5'…TTAA...3' 3'…AATT…5'	unit 200 1,000	Cat. No. E-2101 E-2102

Source : *Thermus ruber 9.*
■ **Reaction Condition** : *AccuCut*™ buffer Blue : 10mM Tris-HCl, 10 mM $MgCl_2$, 10 mM NaCl, 1 mM DTT, pH 8.5, **Incubate at 65℃.**
Storage Buffer : 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration : * 20,000 units/mL.

Heat Inactivation : No.
Isoschizomer : *Mse* I, *Tru*1 I.

Neoschizomer : Unfound

Reactivity on methylated substrate
DNA: Unidentified

Restriction Enzymes

*Mly*113 I (*Nar* I)	▼ 5'…GGCGCC…3' 3'…CCGCGG…5'' ▲	unit 200 1,000	Cat. No. E-1801 E-1802

Source: *Micrococcus lylae 113.*
■ **Reaction Condition**: *AccuCut*™ buffer greeN :
10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6,
Incubate at 37°C.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *5,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Mch* I, *Nar* I, *Nda* I, *Nun* II, *Sse*A I.

Neoschizomer: *Bbe* I (GGCGCC), *Ege* I, *Ehe* I (GGCGCC), *Kas* I (GGCGCC)

Reactivity on methylated substrate
DNA: Unidentified

*Bsp*19 I (*Nco* I)	▼ 5'…CCATGG…3' 3'…GGTACC…5' ▲	unit 500 2,500	Cat. No. E-1381 E-1382

Source: *Bacillus species 19.*
■ **Reaction Condition**: *AccuCut*™ buffer Blue :
10 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 8.5, **Incubate at 37°C.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 5,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Nco* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA: unidentified

*Fau*ND I (*Nde* I)	▼ 5'…CATATG…3' 3'…GTATAC…5' ▲	unit 500 2,500	Cat. No. E-1671 E-1672

Source: *Flavobacterium aquatili ND.*
■ **Reaction Condition**: *AccuCut*™ buffer vlolet :
33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate, 1 mM DTT, pH 7.9, **Incubate at 37°C.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *10,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Nde* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Unidentified

04 Restriction Enzyme

Restriction Enzymes

*Asu*NH I (*Nhe* I)	▼ 5'…GCTAGC… 3' 3'…CGATCG… 5' ▲	unit 600 3,000	Cat. No. E-1201 E-1202

Source: *Actinobacillus suis* NH.
■ **Reaction Condition**: *AccuCut*™ buffer greeN :
10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6,
Incubate at 37℃.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *30,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Nhe* I, *Pst*NH I.

Neoschizomer: *Unfound*

Reactivity on methylated substrate
DNA : Unidentified.

*Cci*N I (*Not* I)	▼ 5'…GCGGCCGC…3' 3'…CGCCGGCG…5' ▲	unit 200 1,000	Cat. No. E-1591 E-1592

Source: *Curtobacterium citreus* N.
■ **Reaction Condition**: *AccuCut*™ buffer vlolet :
33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate,
1 mM DTT, pH 7.9, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 5,000 units/mL.
(Assayed Adenovirus-2 DNA)

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Not* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA: unidentified

*Sfr*274 I (*Pae*R7 I)	▼ 5'…CTCGAG…3' 3'…GAGCTC…5' ▲	unit 3,000 15,000	Cat. No. E-2031 E-2032

Source: *Streptomyces fradiae* 274.
■ **Reaction Condition**: *AccuCut*™ buffer greeN :
10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT,
pH 7.6, **Incubate at 50℃**.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *10,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Abr I, Blu I, Ccr* I, *Mav* I, *Pae*R7 I,
Pan I, *Sla* I, *Xho* I, *Xpa* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Unidentified

Restriction Enzymes

Pst I	5'…CTGCAG…3' 3'…GACGTC…5'	unit 10,000 50,000	Cat. No. E-1951 E-1952

Source: Providencia stuartii.
■**Reaction Condition**: *AccuCut*™ buffer Red : 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃**.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃

Concentration: * 50,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Api* I, *Asp*713 I, *Bsp*B I, *Bsp*63 I, *Hal* II, *Sfl* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by^{m5}CTGCAG/^{m5}CTG^{m5}CAG /CTG^{m5}CAC/CTGC^{m6}AG

Pvu II	5'…CAGCTG…3' 3'…GTCGAC…5'	unit 1,500 7,500	Cat. No. E-1961 E-1962

Source: Proteus vulgaris 84.
■**Reaction Condition**: *AccuCut*™ buffer Orange : 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃

Concentration: *30,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Bav* I, *Dma* I, *Pvu*84 II.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by^{m5}CAGCTG/CAG^{m5}CTG/CAG^{m4}C T G

Rsa I	5'…GTAC…3' 3'…CATG…5'	unit 1,000 5,000	Cat. No. E-1971 E-1972

Source: *Rhodopseudomonas sphaeroides.*
■ **Reaction Condition**: *AccuCut*™ buffer greeN : 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.6, **Incubate at 37℃**.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *10,000 units/mL.

Heat Inactivation: No.
Isoschizomer: *Afa* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by GT^{m6}AC /GTA ^{m4}C.
Not blocked by GTA^{m5}C.

Restriction Enzymes

		unit	Cat. No.
*Psp*124B I (*Sac* I)	▼ 5'…GAGCTC…3' 3'…CTCGAG…5' ▲	1,500 7,500	E-1891 E-1892

Source: *Pseudomonas species* 124B.
■ **Reaction Condition**: *AccuCut*™ buffer Orange : 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: * 15,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Sac* I, *Sst* I.

Neoschizomer: *Ecl*136 II, *Eco*ICR I (GAGCTC).

Reactivity on methylated substrate
DNA : unidentified

		unit	Cat. No.
Sal I	▼ 5'…GTCGAC…3' 3'…CAGCTG…5' ▲	1,000 5,000	E-1981 E-1982

Source: *Streptomyces albus.*
■ **Reaction Condition**: *AccuCut*™ buffer Red : 50mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *20,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Hgi*C III, *Hgi*D II, *Nop* I, *Xci* I.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA : Blocked by G t^{m5}CGAC, GTCG^{m6}AC, Gh^{m5}UCGAC. Not blocked by GTCGA^{m5}C.

		unit	Cat. No.
SfaN I	▼ 5'….GCATCNNNNN…3' 3'….CGTAGNNNNNNNNN…5' ▲	50 250	E-2001 E-2002

Source: *Streptococcus faecalis N.*
■ **Reaction Condition**: *AccuCut*™ buffer Red : 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *1,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: None.

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA: Not blocked by GCAT^{m5}C.

Restriction Enzymes

Sma I	▼ 5'…CCCGGG…3' 3'…GGGCCC…5' ▲	unit 1,000 5,000	Cat. No. E-2051 E-2052

Source: *Serratia marcescens*.
■**Reaction Condition**: *AccuCut*™ buffer vlolet :
33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate,
1 mM DTT pH 7.9, **Incubate at 25℃**.
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *25,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Cfr*J4 I, *Pae*B I, *Psp*AL I.

Neoschizomer: *Cfr*9 I, *Xma* I (CCCGGG)

Reactivity on methylated substrate
DNA : Bolcked by ^{m4}CCCGGG, ^{m5}CCCGGG, C^{m4}CGGG,
CC^{m4}CGGG, CC^{m5}CGGG. Not blocked by
C^{m5}CCGGG

Xba I	▼ 5'…TCTAGA…3' 3'…AGATCT…5' ▲	unit 2,000 10,000	Cat. No. E-2151 E-2152

Source: *Xanthomonas badrii.*
■**Reaction Condition**: *AccuCut*™ buffer Red :
50mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT
pH 7.6, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *25,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: None

Neoschizomer: Unfound

Reactivity on methylated substrate
DNA: Unidentified

Xma I	▼ 5'… CCCGGG…3' 3'… GGGCCC…5' ▲	unit 50 250	Cat. No. E-2161 E-2162

Source: Xanthomonas malvacearum.
■**Reaction Condition**: *AccuCut*™ buffer violet :
33 mM Tris-acetate, 10 mM Mg-acetate, 66 mM K-acetate,
1 mM DTT pH 7.9, **Incubate at 37℃.**
Storage Buffer: 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA,
1 mM 2-mercaptoethanol, 50% glycerol, pH 7.5, Store at -20℃
Concentration: *2,000 units/mL.

Heat Inactivation: Yes. (65℃ for 20 minutes)
Isoschizomer: *Ahy* I, *Cfr*9 I, *Eae*A I, *Psp*A I, *Xcy* I, *Xma*C I.

Neoschizomer: *Sma* I

Reactivity on methylated substrate
DNA : Unidentified

04
Restriction Enzyme