Certificate of Analysis

PstI Restriction Enzyme:

Part No.	Size (units)
R611A	3,000
R611B	15,000

Buffer H (R008A): The Restriction Enzyme 10X Buffer H supplied with this enzyme has a composition of 900mM Tris-HCl (pH 7.5), 500mM NaCl, 100mM $MgCl_2$ and 10mM DTT at 37°C.

BSA in Restriction Enzyme Reactions: All Promega restriction enzyme activity assays are performed with the addition of Acetylated BSA to a final concen-tration of 0.1mg/ml. BSA has been shown to enhance the activity of many restriction enzymes. Promega supplies a tube of Acetylated BSA (Part# R396D, E, or F) with all restriction enzymes and recommends the addition of BSA to a final concentration of 0.1mg/ml to all restriction digestions.

MULTI-CORE™ Buffer (R999A): The MULTI-CORE™ 10X Buffer, which is supplied with enzymes that exhibit >25% activity in this buffer, has a composition of 250mM Tris acetate (pH 7.8), 1M potassium acetate, 100mM magnesium acetate and 10mM DTT. PstI is 25–50% active in MULTI-CORE™ Buffer in 0.1mg/ml Acetylated BSA.

Recognition Sequence : 5 ...C TGCA'G...3
3 ...G'ACGT C...5

Enzyme Storage Buffer: PstI is supplied in 10mM Tris-HCl (pH 7.3), 300mM NaCl, 0.1mM EDTA, 1mM DTT, 0.15% Triton® X-100, 0.5mg/ml BSA and 50% glycerol.

Source: *Providencia stuartii* (1).

Unit Definition: One unit is defined as the amount of enzyme required to completely digest 1µg of lambda DNA in one hour at 37°C in 50µl assay buffer containing Acetylated BSA added to a final concentration of 0.1mg/ml.

Optimal Assay Temperature: 37°C.

Storage Temperature: See the Product Information Label for storage recommendations. Avoid frequent temperature changes. See the expiration date on the Product Information Label.

Assay Information

Overdigests (OD): The overdigestion assay is performed to demonstrate the absence of nonspecific endonucleases and exonucleases. Increasing amounts of enzyme are added to a series of tubes containing substrate DNA. After a 16-hour incubation under appropriate conditions, the maximum number of units giving a clear, sharp, normal banding pattern is determined by agarose gel/ethidium bromide electrophoresis. The minimum number of units of PstI required to meet these criteria using 1µg of lambda DNA as the substrate and the recommended digestion conditions (50µl reaction, Buffer H, 37°C) is 50 units.

Cut:Ligation:Recut (C:L:R): The ligation assay is used to determine the functional purity of the DNA after restriction enzyme digestion. Substrate DNA is completely digested with a fourfold excess of the restriction enzyme in the appropriate assay buffer, ligated with T4 DNA Ligase and recut with the same restriction enzyme. Cut, ligated and recut DNAs are analyzed by agarose gel/ethidium bromide electrophoresis. A normal banding pattern indicates intact 5 and 3 termini as well as the absence of contaminating nucleases. The minimum C:L:R ratio for this enzyme is 100:90:90.

Blue/White Cloning Qualified: This assay is performed to determine the incidence of false positives (2). A pGEM®-Z series Vector containing a unique PstI site is digested with 25 units of PstI per 5µg of DNA for 2 hours at 37°C. Linearized plasmid is purified using Wizard® *Plus* Minipreps DNA Purification System and ligated at 25°C for 30 minutes. Competent JM109 cells are transformed with ligated plasmids and plated on X-Gal/IPTG/Amp_{100} plates. A minimum of 400 colonies are counted. White colonies result from transformation with ligated plasmids with damaged ends. These white colonies represent the number of false positives expected in a typical cloning experiment. Enzymes that generate overhangs must produce fewer than 2% white colonies and blunt cutting enzymes must produce fewer than 5% white colonies.

Product Information

Buffer Activity:

Percent (%) activity in 4-CORE® Buffer System

A	B	C	D	H	MC
10–25	50–75	50–75	50–75	**100**	**25–50**

Key: A–D 4-CORE® Buffer System
MC MULTI-CORE™ Buffer

Heat Inactivation:

	Heat Inactivated
PstI	+

Key: + 95% inactivation by incubation at 65°C for 15 minutes
– no inactivation
+/– partial inactivation

Frequency of Cutting:

λ	Ad-2	fX174	pUC18	M13mp18	pBR322
28	30	1	1	1	1

Methylation:

	dam	dcm	CpG	CpNpG
PstI	i	i	i	s

Key: s = sensitive to this methylation
i = insensitive to this methylation
s(ol) = overlapping (sensitive when restriction site overlaps methylation sequence)
n/a = information not available

References

1. Smith, D.I., Blattner, F.R. and Davies, J. (1976) *Nucl. Acids Res.* **3**, 343–53.
2. Hung, L. *et al.* (1991) *Promega Notes* **33**, 12.

Signed by:

R. Wheeler, Quality Assurance

PstI

REF R6111 LOT 0000254398
-30°C -10°C 2018-11-12
Conc: 10u/µl
3,000u
Dispensed Lot#: 0000251064

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Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399 USA

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Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

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Promega

Usage Information

Introduction

Restriction enzymes, also referred to as restriction endonucleases, are enzymes which recognize short, specific (often palindromic) DNA sequences. They cleave double-stranded DNA (dsDNA) at specific sites within or adjacent to their recognition sequences. Most restriction enzymes (REs) will not cut DNA that is **methylated** on one or both strands of their **recognition site**, although some require substrate methylation.

Each restriction enzyme has specific requirements to achieve optimal activity. Ideal storage and assay conditions favor the most activity and highest fidelity in a particular enzyme's function. Conditions such as temperature, pH, enzyme cofactor(s), salt composition and ionic strength affect enzyme activity and stability. Two buffers usually accompany each of Promega's restriction enzymes. One buffer is the optimal reaction buffer which may be from the **4-CORE® System** (Reaction Buffers A, B, C, D) or one of the other optimal buffers (Reaction Buffers E–L), and the other is the MULTI-CORE™ Buffer. The supplied optimal buffer always yields 100% activity for the enzyme it accompanies, and serves as the specific reaction buffer for individual digests with that enzyme. **The MULTI-CORE™ Buffer**, which is designed for broad compatibility with many REs, is provided with enzymes that have 25% or greater activity in this buffer. The MULTI-CORE™ Buffer is useful for multiple digests because it generally yields more activity for more enzyme combinations than any of the other buffers, but sometimes with a compromise in activity. Multiple digests using REs with significantly different buffer requirements may require a sequential reaction with the addition of RE buffer or salt before the second enzyme is used.

DNA Substrate Considerations

DNA substrates commonly used for restriction enzyme digestion include DNA from bacteriophage lambda, bacterial plasmid DNA and genomic DNA. Lambda DNA is a linear DNA form that is an industry standard for measuring and expressing unit activity for many restriction enzymes. Compared to linear DNA, intact supercoiled plasmid DNA (and DNAs with a large number of the target restriction site) require more units of enzyme (two- to tenfold) per microgram than the DNA used in the enzyme's activity assay.

PCR products and oligonucleotides are relatively small compared with DNA used for defining RE units. Therefore, when using these substrates in a restriction digest, it is essential to take into consideration the molar concentration of enzyme recognition sites and not just the mass of DNA. Also, some REs require flanking bases surrounding the core RE recognition site. This is problematic when it is necessary to cut an oligonucleotide or a fragment of DNA with an RE site near its end. When PCR cloning strategies include the use of primers containing an RE site, care is necessary in designing the primer with adequate DNA surrounding the core RE recognition sequence.

In addition to the form and original source of the DNA, the purity is another factor that must be considered. Depending on the purification method and the handling of the DNA, it may contain varying amounts of contaminants that affect restriction enzyme digestion and analysis. Contaminants may include other types of DNA, nucleases, salts and inhibitors of restriction enzymes. The effect of a contaminant on an RE digest is generally dose-dependent: i.e., the inhibitory effects will increase with the volume of DNA added to the restriction enzyme reaction. Relatively pure DNA is required for efficient restriction enzyme digestion. Contaminating nucleases are usually activated only after the addition of salts (e.g., restriction enzyme buffer) to the DNA solution. Therefore, appropriate control reactions should always be run in parallel with the restriction digest. Buffer solutions containing EDTA in low concentrations (1mM) are often used to protect DNA from nuclease degradation during storage, but the EDTA can interfere with restriction enzyme digestion if the final concentration of EDTA in the reaction is too high. This situation usually results when the concentration of the substrate DNA is low and it is necessary to use a large volume of DNA in the digest. In such cases, it is best to concentrate the DNA (e.g., by ethanol precipitation). The organic solvents, salts, detergents and chelating agents that are sometimes used during the purification of DNA can also interfere with restriction enzyme activity if they carry over into the final DNA solution. Dialysis and/or ethanol precipitation with 2.5M ammonium acetate (final concentration before adding ethanol) followed by drying and resuspension can remove many of these substances. While relatively pure DNA is required for efficient restriction enzyme digestion, addition of acetylated BSA to a final concentration of 0.1mg/ml can sometimes improve the quality and efficiency of enzyme assays containing impure DNA and we recommend that it be included in all digests.

Enzyme Storage, Handling and Use

Maintain the sterility of reagents used in the RE digest as well as any tools (e.g., tubes, pipette tips) used with those reagents. Restriction enzymes should be stored in a nonfrost-free freezer, except for a brief period during use, when they should be kept on ice. The restriction enzyme is usually the last reagent added to a reaction, to ensure that it is not exposed to extreme conditions. When many similar digests are being prepared, it may be convenient to create premixes of common reagents.

Before assembling the restriction digest, thoroughly mix each component to be added to the reaction and then centrifuge the tubes of reagents briefly to collect the contents in the bottom of the tube. The reaction components should also be mixed after addition of the enzyme to the digest. While high salt buffers and glycerol-containing reagents are difficult to mix, all solutions containing restriction enzymes must be mixed gently to avoid inactivating the enzyme.

Setting up a Restriction Enzyme Digest

An analytical scale restriction enzyme digest is usually performed in a volume of 20µl on 0.2–1.5µg of substrate DNA, using a two- to tenfold excess of enzyme over DNA. If an unusually large volume of DNA or enzyme is used, aberrant results may occur and may or may not be readily recognized. The following is an example of a typical RE digest. In a sterile tube, assemble in order:

sterile, deionized water	16.3µl
RE 10X Buffer	2µl
Acetylated BSA, 10µg/µl	0.2µl
DNA, 1µg/µl	1µl

Mix by pipetting, then add:

Restriction Enzyme, 10u/µl	0.5µl
final volume	20µl

Mix gently by pipetting, close the tube and centrifuge for a few seconds in a microcentrifuge. Incubate at the optimum temperature for 1–4 hours.

Add 4µl of 6X loading buffer and proceed to gel analysis. Note that overnight digests are usually unnecessary and may result in degradation of the DNA.

Experimental Controls

Experimental controls are necessary to identify, understand and explain problems or inconsistencies in results. The following controls are commonly used in parallel with RE digests: (i) uncut experimental DNA, (ii) digest of commercially supplied control DNA, (iii) no-enzyme "mock" digest, (iv) 1 or 2 different size markers in more than one lane per gel (i.e., different locations).

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