Manual

Endonuclease VIII Kit

For Research Use Only. Not for use in diagnostic procedures.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

Endonuclease VIII Kit

Contents

Manual

Endonuclease VIII Kit

1. Introduction

E. coli Endonuclease VIII is a bifunctional DNA glycosylase with DNA N-glycosylase and AP lyase activities. The N-glycosylase activity releases damaged pyrimidines from double-stranded DNA generating an apurinic (AP) site. The AP lyase activity cleaves the DNA phosphodiester backbone at AP sites via β- and δ-elimination, creating a one nucleotide gap with 5′ and 3′ phosphate termini. Damaged bases recognised and removed by Endonuclease VIII include thymine glycol, uracil glycol, urea, 5,6-dihydroxythymine, 5-hydroxy-5-methylhydantoin, 5-hydroxy-6-hydrothymine, 5,6-dihydrouracil and alloxan. Although Endonuclease VIII is like Endonuclease III, Endonuclease VIII has both β and δ lyase activity while Endonuclease III has only β lyase activity..

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
Endonuclease VIII Kit	1,000 units	30270	Endonuclease VIII (10 U/µL)	F836129-1	100 µL
			Endonuclease VIII Reaction Buffer, 10X	F836075-1	1 mL
	10,000 units	30271	Endonuclease VIII (10 U/µL)	F836129-2	1 mL
			Endonuclease VIII Reaction Buffer, 10X	F836075-2	5 mL

3. Product specifications

Storage: Store at -20 °C in a freezer without a defrost cycle.

Storage buffer: Endonuclease VIII is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 8.0), 250 mM NaCl, 0.1 mM EDTA.

Endonuclease VIII Reaction Buffer, 10X: 100 mM Tris-HCl (pH 8.0), 750 mM NaCl, 10 mM EDTA.

Unit definition: One unit is the amount of enzyme required to cleave 1 pmol of 72 base pair oligonucleotide duplex containing a single abasic* site on each strand in 1 hour at 37 °C in 25 µL in 1x Endonuclease VIII reaction buffer containing 10 pmol of oligonucleotide duplex.

*Abasic site is created by including Uracil N-Glycosylase (UNG) concurrently with the Endonuclease VIII.

Contaminating activity assays: Endonuclease VIII is free of detectable Endonuclease, Exonuclease and RNase activities.

Purity: Endonuclease VIII is assessed as ≥95% pure by SDS-Polyacrylamide gel electrophoresis.

Heat inactivation: Endonuclease VIII can be inactivated by heating at 75 °C for 10 minutes.

4. References

Kropachev, K. Y. *et al.* (2006) *Biochemistry*, 45, 12039-12049. The catalytic mechanism of *E. coli* endonuclease VIII: Roles of the intercalation loop and the zinc finger.

Pflaum, M. *et al.* (1998) *Free Rad. Res,* 29, 585-594. Alkaline elution. DNA oxidation products determined with repair endonucleases in mammalian cells: Types, basal levels and influence of cell proliferation.

Jiang, D. *et. al*. (1997) *J. Biol. Chem*., 272, 32230-32239. Characterization of *Escherichia coli* Endonuclease VIII.

Hartwig, *et al*. (1996). *Toxicol. Lett*, 88, 85-90. Alkaline unwinding. Sensitive analysis of oxidative DNA damage in mammalian cells.

Collins, A. *et al.* (1996). *Environ. Health Perspect,* 104, 465-469. Comet assay. Direct enzymatic detection of endogenous oxidative base damage in human lymphocyte DNA.

Demple, B, and Harrison, L. (1994) *Annu. Rev. Biochem*, 63, 915-48 (see page 923). Repair of Oxidative Damage to DNA: Enzymology and Biology.

Hatahet, Z. *et al.* (1994) *J. Biol. Chem*., 269, 18814-18820. New substrates for old enzymes.

Dizdaroglu, M. *et al*. (1993) *Biochemistry* 32, 12105-12111. Substrate specificity of the *Escherichia coli* endonuclease III: Excision of thymine- and cytosine-derived lesions in DNA produced by radiation-generated free radicals.

Collins, A. *et al*. (1993). *Carcinogenesis*, 14, 1733-1735. Comet assay. A simple technique for quantitation of low levels of DNA damage in individual cells.

Singh, N. *et al.* (1988) *Exp. Cell. Res*, 175, 184-191. Comet assay. Oxidative damage to DNA: Do we have a reliable biomarker?

@LGCBiosearch | biosearchtech.com

GEN/1043/EK/0123

For Research Use Only. Not for use in diagnostic procedures.

bsi. ISO 13485 Medical Devices Quality Management
FM 598408

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC