

PspGI



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R0611S 002120814081



R0611S

1,000 units 10,000 U/ml Lot: 0021208
RECOMBINANT Store at -20°C Exp: 8/14

Recognition Site:

5'...CCWGG...3'
3'...GGWCC...5'

Single Letter Code: W = A or T

New Reaction Buffer

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Source: An *E. coli* strain that carries the cloned PspGI gene from *Pyrococcus* species strain GI-H (H. W. Jannasch)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 4.

Reaction Conditions: 1X NEBuffer 4.
Incubate at 75°C.

1X NEBuffer 4:

50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of T7 DNA in 1 hour at 75°C in a total reaction volume of 50 µl.

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Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

Quality Control Assays

Ligation: After 10-fold overdigestion with PspGI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

Exonuclease Activity: Incubation of 50 units of enzyme with 1 µg sonicated ³H DNA (10⁵ cpm/µg) for 4 hours at 75°C in 50 µl reaction buffer released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	100%
NEBuffer 3	50%
NEBuffer 4	100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

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Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Notes: PspGI is a highly thermostable isoschizomer of BstNI and EcoRII.

Activity at 85°C is 2-3 fold greater than at 75°C. At 95°C, PspGI has a half-life of 2 hours.

Blocked by *dcm* methylation.

Incubation at 37°C results in 10% activity.

Overdigestion of > 20 units of PspGI per µg DNA and incubation times > 2 hours are **not** recommended.

Companion Products:

dam/*dcm* Competent *E. coli*
#C2925H 20 transformation reactions
#C2925I 24 transformation reactions

U.S. Patent No. 5,849,558

CERTIFICATE OF ANALYSIS

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