

**FspI**

NEW ENGLAND  
BioLabs

1-800-632-7799  
info@neb.com  
www.neb.com

R0135S 058120614061

**R0135S**

500 units    5,000 U/ml    Lot: 0581206

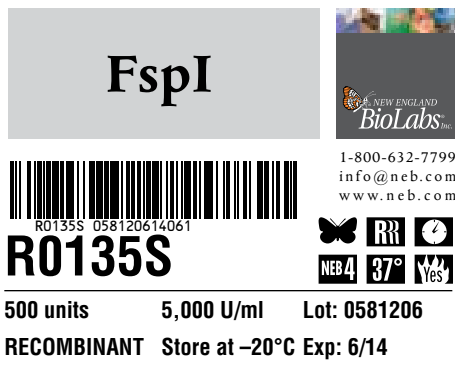
**RECOMBINANT**    Store at  $-20^{\circ}\text{C}$     Exp: 6/14

**Recognition Site:**

5'... T G C<sup>▼</sup> G C A... 3'  
3'... A C G<sup>▲</sup> C G T... 5'

**Source:** An *E. coli* strain that carries the cloned FspI gene from *Fischerella* species (ATCC 29114)

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 300 µg/ml BSA and 50% glycerol.



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**Reagents Supplied with Enzyme:**  
10X NEBuffer 4

**Reaction Conditions:** 1X NEBuffer 4.  
Incubate at 37°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 λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

**Diluent Compatibility:** Diluent Buffer C  
250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA,  
1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/  
ml BSA and 50% glycerol  
(pH 7.4 @ 25°C).

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**Quality Control Assays**

**Ligation:** After 5-fold overdigestion with FspI, > 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.

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of DNA and 100 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

**Exonuclease Activity:** Incubation of 100 units of enzyme with 1 µg sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

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**Enzyme Properties**

**Activity in NEBuffers:**


NEBuffer 1	10%
NEBuffer 2	75%
NEBuffer 3	10%
NEBuffer 4	<b>100%</b>

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

**Survival in a Reaction:** A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

**Heat Inactivation:** 25 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

**Note:** Cleavage of mammalian genomic DNA is blocked by CpG methylation.

 = Time-Saver™ Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS