

Tsp45I



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



R0583S 003121014101

R0583S



200 units Lot: 0031210 Exp: 10/14

4,000 U/ml Store at -20°C

Recognition Site:

5'...**▼**G T S A C ... 3'
3'... C A S T G **▲**... 5'

Single Letter Code: S = C or G

Source: *Thermus* species YS45 (R.A.D. Williams)

2X More Units

Tsp45I



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



R0583S 003121014101

R0583S



200 units Lot: 0031210 Exp: 10/14

4,000 U/ml Store at -20°C

Recognition Site:

5'...**▼**G T S A C ... 3'
3'... C A S T G **▲**... 5'

Single Letter Code: S = C or G

Source: *Thermus* species YS45 (R.A.D. Williams)

2X More Units

Supplied in: 20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 1, 100X BSA.

Reaction Conditions: 1X NEBuffer 1, supplemented with BSA. **Incubate at 65°C.**

1X NEBuffer 1:

10 mM Bis Tris Propane-HCl
10 mM MgCl₂
1 mM DTT
pH 7.0 at 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 65°C in a total reaction volume of 50 µl.

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

Supplied in: 20 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:

10X NEBuffer 1, 100X BSA.

Reaction Conditions: 1X NEBuffer 1, supplemented with BSA. **Incubate at 65°C.**

1X NEBuffer 1:

10 mM Bis Tris Propane-HCl
10 mM MgCl₂
1 mM DTT
pH 7.0 at 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 65°C in a total reaction volume of 50 µl.

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

Quality Control Assays

Ligation: After 10-fold overdigestion with Tsp45I, > 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 20 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction produced in one hour with one unit of enzyme.

Exonuclease Activity: Incubation of 12 units for 4 hours at 65°C in 50 µl assay buffer with 1 µg sonicated ³H DNA (10⁵ cpm/µg) released < 0.1% radioactivity.

Quality Control Assays

Ligation: After 10-fold overdigestion with Tsp45I, > 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 20 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction produced in one hour with one unit of enzyme.

Exonuclease Activity: Incubation of 12 units for 4 hours at 65°C in 50 µl assay buffer with 1 µg sonicated ³H DNA (10⁵ cpm/µg) released < 0.1% radioactivity.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	25%
NEBuffer 3	0%
NEBuffer 4	75%

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 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No.

Notes: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Incubation at 37°C results in 10% activity.

U.S. Patent No. 5,866,422

CERTIFICATE OF ANALYSIS

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	25%
NEBuffer 3	0%
NEBuffer 4	75%

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 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No.

Notes: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Incubation at 37°C results in 10% activity.

U.S. Patent No. 5,866,422

CERTIFICATE OF ANALYSIS