

CERTIFICATE OF ANALYSIS

TaaI (HpyCH4III)

#ER1361 200 u

Lot: Expiry Date:

5'...**A C N↓G T**...3'

3'...**T G↑N C A**...5'

Concentration: 10 u/μl
Source: *Thermus aquaticus* Vn 4-311
Supplied with: 1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer Tango™ (for 100% TaaI digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

Incubation temperature

65°C*.

Unit Definition

One unit is defined as the amount of TaaI required to digest 1 μg of lambda DNA in 1 hour at 65°C in 50 μl of recommended reaction buffer.

Dilution

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

Double Digests

Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer.

Please refer to the Fermentas Catalog or go to www.fermentas.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer

TaaI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

* Incubation at 37°C results in 10% activity.

Recommended Protocol for Digestion

- Add:

| | |
|------------------------------|---------------|
| nuclease-free water | 16 μ l |
| 10X Buffer Tango™ | 2 μ l |
| DNA (0.5-1 μ g/ μ l) | 1 μ l |
| Taal | 0.5-2 μ l |
- Mix gently and spin down for a few seconds.
- Incubate at 65°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

| | |
|----------------------|--------------------------------------|
| PCR reaction mixture | 10 μ l (~0.1-0.5 μ g of DNA) |
| nuclease-free water | 18 μ l |
| 10X Buffer Tango™ | 2 μ l |
| Taal | 1-2 μ l |
- Mix gently and spin down for a few seconds.
- Incubate at 65°C for 1-16 hours.

Thermal Inactivation

Taal is not inactivated by incubation at 80°C for 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
 - stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
 - extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
 - dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
 - check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **QUALITY CONTROL ASSAY DATA**
see back page

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

| B | G | O | R | Tango™ | 2X Tango™ |
|------|------|------|--------|--------|-----------|
| 0-20 | 0-20 | 0-20 | 50-100 | 100 | 100 |

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: may overlap – cleavage impaired.

EcoKI: may overlap – effect not determined.

EcoBI: may overlap – effect not determined.

Stability during Prolonged Incubation

A minimum of 0.2 units of enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 65°C.

Compatible Ends

Hpy188I

Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|-----|-------|--------|-------|----------|----------|------------|
| 187 | 15 | 14 | 8 | 8 | 7 | 31 |

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Taal (10 u/µg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with Taal approximately 90% of the digested DNA fragments can be ligated at a 5'-termini concentration of 1.9 µM. More than 90% of these sites can be recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Taal for 4 hours.

Quality authorized by:



Jurgita Zilinskiene

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

For **QUALITY CONTROL ASSAY DATA** see back page

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