Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

DpnI

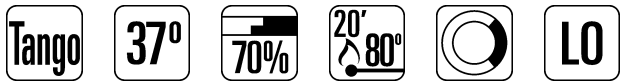
#ER1701 500 u

Lot: ___ **Expiry Date: _**

5'...**G m6A↓ T C** ...3'
3'...**C T↑m6A G** ...5'

Concentration: 10 u/µl
Source: *E.coli* that carries the cloned *dpnIR* gene from *Diplococcus pneumoniae* G41
Supplied with: 1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials. BSA included

ISO 9001 ISO 14001
www.fermentas.com

RECOMMENDATIONS

1X Buffer Tango™ (for 100% DpnI digestion)
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of DpnI required to digest 1 µg of pBR322 DNA (*dam* methylated) in 1 hour at 37°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
DpnI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 400 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

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Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer Tango™	2 µl
DNA (0.5-1 µg/µl)	1 µl
DpnI	0.5-2 µl

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Thermal Inactivation

DpnI is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

B	G	O	R	Tango™	2X Tango™
100	100	50-100	50-100	**100**	50-100

Methylation Effects on Digestion

Dam: does not cut *dam*⁻ DNA.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – effect not determined.

Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of pBR322 DNA in 16 hours at 37°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
116	0	22	15	15	15	7

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

Note

- DpnI requires the presence of N6-methyladenine within the recognition sequence to cleave DNA.
- DNA purified from a *dam*$^+$ strain will be a substrate for DpnI.
- DpnI will only cleave fully-adenomethylated *dam* sites. Hemi-adenomethylated *dam* sites DpnI cleaves 60X more slowly.
- DpnI, Bsp143I and MboI all recognize the same sequence but have different methylation sensitivities and cleavage sites.

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with DpnI (10 u/µg pBR322 DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg pBR322 DNA x 17 hours) with DpnI, more than 70% of the digested pBR322 DNA fragments can be ligated at a 5'-termini concentration of 2.3 µM. More than 95% 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 DpnI for 4 hours.

Quality authorized by:  Jurgita Zilinskiene