

MBRE017
Nco I
Components

Reagents provided	MBRE017			
	250 Units	500 Units	1000 Units	5000 Units
Nco I	25 µl	50 µl	100 µl	500 µl
10X HiBuffer H4	25 µl	50 µl	0.1 ml	0.5 ml
10X HiBuffer DB	25 µl	50 µl	0.1 ml	0.5 ml
Diluent E Buffer	25 µl	50 µl	0.1 ml	0.5 ml

NOTE: BSA included in all Reaction Buffer

Source: A *E. coli* strain that carries the Hind III gene from *Nocardia corallina*

Recognition Sequence:


Concentration: 10 U/µl

Unit Definition:

1 u is defined as the amount of enzyme that is required to digest 1µg of DNA in 1 hour at 37°C in 50µl of assay buffer.

Enzyme	Optimum reaction temperature (°C)	Thermal Inactivation (°C)	% activity of Buffers				
			H1	H2	H3	H4	H5
Nco I	37	65	75	75	100	100	100

Reaction Buffer:
10X HiBuffer H4 :

10mM Tris-HCl (pH 8.5 at 30°C), 10mM MgCl₂, 100mM KCl, and 100 µg/ml BSA.

Note: Incubate at 37°C.

Storage Buffer:

10mM Tris-HCl (pH 7.5), 300mM NaCl, 0.1mM EDTA, 7mM 2-mercaptoethanol, 200µg/ml BSA and 50% glycerol. Store at -20°C.

NOTE: 10X HiBuffer DB is provided for double digestion.

Quality Control Assays:
Ligation / Recutting Assay:

After 10-fold over digestion with Nco I, more than 90% of the DNA fragments can be ligated and recut.

Over digestion Assay:

An unaltered banding pattern was observed after 1µg of DNA was digested with 20U of Nco I for 16

hours at 37°C.

Example of Digestion conditions:

- Enzyme concentration : 1 Unit
- Lambda 0.3 mg/ml : 3.33 µl (1 µg DNA)
- 10X HiBuffer H4 : 5 µl
- Nuclease free water : upto 50 µl

Note:

- Total reaction volume is dependent on the experiment
- The amount of enzyme to be used is dependent on the DNA template
- For plasmid DNA, 5-10X more enzyme is required
- High enzyme concentration may result in **Star activity**

Storage conditions: Nco I should be stored at -20°C.

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