DNA Blunting Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR1101101	100 rxn of 25 µl	125 µl 20× Blunting Enzyme Mix 250 µl 10× Blunting Buffer 25 µl 10 mM dNTP Mix

COMPONENT	COMPOSITION
Blunting Enzyme Mix	20× Blunting Enzyme Mix, in storage buffer containing 50% (v/v) glycerol.
Blunting Buffer	Optimized 10× Blunting Buffer for rapid blunting reaction.
10 mM dNTP Mix	dNTP Mix containing 10 mM dATP, dCTP, dGTP, dTTP in water.
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Rapid blunting reaction
- Excellent performance in blunt-ended ligation
- Ready-to-use blunted product in subsequent ligation

APPLICATIONS

- Blunting and phosphorylation of double-stranded DNA
- Preparation of restriction enzyme digested DNA or sheared DNA for blunt-ended ligation

DESCRIPTION

biotechrabbit™ DNA Blunting Kit shows excellent performance in blunting and phosphorylation of double-stranded DNA. The kit is compatible with a wide variety of dsDNA templates such as restriction enzyme digested DNA fragments, plasmid DNA, PCR products with dA-overhang, sheared or nebulized DNA. The kit is optimized for rapid blunting reaction.

PROTOCOL

Blunting reaction

- Scale the reaction volume up as required.
- Prepare the blunting reaction as shown below:

COMPONENT	VOLUME	FINAL CONCENTRATION
10× Blunting Buffer	2.5 µl	1×
Purified DNA	Variable	up to 5 µg
10 mM dNTP Mix	0.25 µl	0.1 mM
20× Blunting Enzyme Mix	1.25 µl	1×
Nuclease free water	Variable	
Total volume	25 µl	

- Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
- Incubate at 25°C (room temperature) for 15 minutes.
- For better efficiency or sheared/nebulized DNA extend the incubation time to 30 min.
- Heat inactivate the enzyme at 70°C for 10 minutes.
- Blunted DNA can be directly used in ligation step (overnight ligation suggested).

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay
Purified double digested sticky-end DNA is blunted, ligated and successful ligation is confirmed.

Exonuclease Activity
Linearized lambda/HindIII DNA fragments are incubated with the enzyme in a 50 µl reaction mixture for 4 h at 37°C. No DNA degradation observed.

Endonuclease/Nick Activity
Supercoiled plasmid DNA is incubated with the enzyme in a 50 µl reaction mixture for 4 h at 37°C. No conversion of covalently closed circular DNA to nicked DNA detected.

Contamination with *E. coli* DNA
Absence of *E. coli* genomic DNA is confirmed by qPCR using a sample of the enzyme and specific primers targeting the *E. coli* 16S rRNA gene. No contamination detected.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS
For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS
- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

CONTACT BIOTECHRABBIT

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Certified Quality-System mdc ISO 13485 ISO 9001

Legal Disclaimer and Product Use Limitation

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valid from 09.04.2019