Quick Blunting™ Kit

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

E1201S 007130215021

E1201S

20 reactions **Lot: 0071302** **Exp: 2/15**

RECOMBINANT **Store at –20°C**

Description: The Quick Blunting Kit is used to convert DNA with incompatible 5´or 3´overhangs to 5´phosphorylated, blunt-ended DNA for efficient blunt-end ligation into DNA cloning vectors. DNA is blunted using T4 DNA polymerase (NEB #M0203) which has both 3´→ 5´ exonuclease activity and 5´→ 3´ polymerase activity. T4 Polynucleotide Kinase (NEB #M0201) is included in the enzyme mix for phosphorylation of the 5´ends of blunt-ended DNA for subsequent ligation into a cloning vector. This kit is optimized for blunting up to 5 µg of DNA in a single reaction.

Applications:

- Prepare sheared, nebulized or restriction enzyme digested DNA for blunt-ended ligation into a plasmid, cosmid, fosmid or BAC vector
- Prepare PCR products for efficient blunt-end cloning

Advantages:

- Fast - Restriction enzyme digested DNA is blunted in less than 30 minutes
- Convenient - Reactions are performed at room temperature in a ready-to-use mix
- Flexible - Suitable for restriction enzyme digested DNA, sheared or nebulized DNA or PCR product

Kit Components:

Blunting Enzyme Mix	25 µl
10X Blunting Buffer	500 µl
1 mM Deoxynucleotide Solution Mix (dNTP Mix)	100 µl

Blunt Enzyme Mix supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Triton X-100 and 50% Glycerol.

1X Blunting Buffer:
100 mM Tris-HCl
50 mM NaCl
10 mM $MgCl_2$
0.025% Triton X-100
5 mM dithiothreitol
pH 7.5 at 25°C

Blunting Protocol:

Reaction volume may be scaled up or down as necessary.

1. Mix the following components in a sterile microfuge tube:

Purified DNA (up to 5 µg)	1–19 µl
10X Blunting Buffer	2.5 µl
1 mM dNTP Mix	2.5 µl
Blunt Enzyme Mix	1.0 µl
Sterile dH_20	variable
Total volume	25 µl

2. Reactions containing restriction enzyme digested DNA are incubated at room temperature for 15 minutes. Reactions with sheared/nebulized DNA or PCR products* are incubated at room temperature for 30 minutes.
3. Immediately inactivate enzyme in the blunting reaction by heating at 70°C for 10 minutes.
4. Proceed directly to the ligation step using the Quick Ligation Kit (NEB #M2200) or standard T4 DNA Ligase (NEB #M0202). Blunt ligation reactions using standard T4 DNA Ligase should be incubated overnight at room temperature.

* see usage notes

Usage Notes:

- PCR generated DNA must be purified before blunting by using a commercial purification kit, phenol extraction/ethanol precipitation, or gel electrophoresis.
- Restriction enzyme digested DNA can be blunted directly without purification. The Blunt Enzyme Mix has been optimized in Blunting Buffer, but is also active in NEBuffers 1,2,3 and 4, as well as BamHI, EcoRI and DpnII unique buffers when supplemented with dNTPs and dithiothreitol. There is a small reduction in ligation fidelity in these buffers. Transformation efficiency is lowest in NEBuffer 1 where the total yield is about 50% of optimum.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

- ATP is not necessary for T4 Polynucleotide Kinase activiity in the this kit. The dATP and dTTP in the dNTP mix act as phosphate donors.

The blunted reaction must be purified prior to phosphatase treatment by using a commercial purification kit, phenol extraction/ethanol precipitation or gel electrophoresis.

Quality Controls

The Quick Blunting Kit is tested with a pUC19 derived construct that is digested with ApaI and BamHI to produce 5´ and 3´overhangs. This construct is blunted, ligated and transformed into NEB 5-alpha Competent *E. coli* (NEB #C2991) and spread on plates containing Xgal, IPTG and ampicillin. Blue colonies indicate precise 5´overhang fill-in and 3´overhang removal of the plasmid DNA.

Companion Products Sold Separately:

Quick Ligation™ Kit	
#M2200S	30 reactions
#M2200L	150 reactions
T4 DNA Ligase	
#M0202S	20,000 units
#M0202L	100,000 units
#M0202T	20,000 units
#M0202M	100,000 units

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