

USB® Blunt-IT™ Repair Kit

- Transform sticky ends of DNA into blunt-ends in as little as 15 minutes
- Blunted products can be directly ligated with the USB Ligate-IT™ Rapid Ligation Kit
- Blunt-IT can be performed directly in restriction enzyme digests

How it works

Many cloning reactions require blunt-end ligation when compatible restriction enzyme sites cannot be used. The Blunt-IT Repair Kit transforms DNA sticky ends into cloning-capable, blunt-ended DNA. The kit uses the Klenow fragment of *E. coli* DNA Polymerase I to fill in 5' overhanging ends of double-stranded DNA with dNTPs⁽¹⁾ and to digest 3' overhanging ends. The Blunt-IT Repair Kit includes Blunt-IT Klenow Enzyme, 10X Blunt-IT Reaction Buffer, dNTP Nucleotide Mix, Nuclease-Free Water and Stop Solution. All of the components are in a ready-to-use format. The Blunt-IT Repair Kit is compatible with most restriction enzyme digestion buffers, allowing reactions to be performed in a single tube.

Fast and convenient DNA ligation

With the Blunt-IT Repair Kit, the resulting DNA can be directly ligated using the USB Ligate-IT Rapid Ligation Kit [PN 78400/10]. By combining these kits, DNA can be made blunt and ligated into a vector or to linkers in as little as 30 minutes. To improve ligation efficiency, blunt-ended DNA can be purified by phenol extraction, ethanol precipitation, column purification, or gel electrophoresis. Blunt-ended vectors should be treated with rSAP [PN 78390] to dephosphorylate the ends to reduce background.

Applications

1. Blunt-end ligation
2. Adapter insertion
3. Removal of 3' overhangs prior to *in vitro* transcription

Storage buffer:

50 mM potassium phosphate (pH 7.0), 1.0 mM DTT, and 50% glycerol.

Shipping and storage:

Shipped on dry ice. Store at -20°C.

Tested User Friendly™ functional test:

pUC19 plasmid is restriction digested independently with Xba I (5' overhang ends) and Pst I (3' overhang ends) to disrupt the *lacZα* gene. After purification, the vectors are repaired using the Blunt-IT Repair Kit. The blunt-ended plasmids are directly ligated using Ligate-IT Rapid Ligation Kit and transformed. Transformants are plated in the presence of an antibiotic and X-Gal [PN 10077] for blue/white selection. Kit exceeds 90% efficiency as measured by the percent of disrupted *lacZα* plasmids (white colonies).

Functionally Tested 10X Blunt-IT Reaction Buffer (1 ml included):

0.5 M Tris-HCl (pH 7.5), 0.1 M MgCl₂, 10 mM DTT, 0.5 mg/ml BSA (nuclease-free).

Stop Solution (100 µl included):

0.5 M EDTA

Kit components

	25 reaction pack size	100 reaction pack size
Blunt-IT Klenow Enzyme (5 units/µl)	25 µl	100 µl
10X Blunt-IT Reaction Buffer	500 µl	500 µl
2 mM dNTP Nucleotide Mix (dG, dA, dT, dC)	25 µl	100 µl
Nuclease-Free Water	1 ml	1 ml
Stop Solution	100 µl	100 µl

Protocol to generate blunt ends:

- In a 1.5 ml tube combine:
 - 0.1 to 4 µg Restriction-digested DNA
 - 2 µl 10X Blunt-IT Reaction Buffer
 - 1 µl 2 mM dNTP Nucleotide Mix
 - 1 µl Blunt-IT Klenow Enzyme
 - to 20 µl Nuclease-Free Water
- Mix by gently pipetting. Centrifuge briefly to collect liquid at bottom of tube.
- Incubate for 15 minutes at room temperature (20-25°C).
- Stop the reaction with 1 µl Stop Solution or heat for 10 minutes at 75°C.
- Blunt-ended DNA can be used directly or can be purified for use in ligation reactions using the Ligate-IT Rapid Ligation Kit [PN 78400/10] or T4 DNA Ligase [PN 70005].

Protocol to generate blunt ends in Restriction Digestion

Buffers:

Following restriction digestion and heat inactivation of the restriction enzyme:

- In a restriction enzyme reaction tube containing 0.1 to 4 µg DNA in 20 µl or less volume, add the following:
 - 1 µl 2 mM dNTP mix
 - 1 µl Blunt-IT Klenow Enzyme
 - to 20 µl Nuclease-Free Water
- Mix by gently pipetting. Centrifuge briefly to collect liquid at bottom of tube.
- Incubate for 15 minutes at room temperature (20-25°C).
- Stop the reaction with 1 µl Stop Solution or heat for 10 minutes at 75°C.
- Blunt-ended DNA can be used directly or can be purified for use in ligation reactions using the Ligate-IT Rapid Ligation Kit [PN 78400/10] or T4 DNA Ligase [PN 70005].

References:

- Telford, J. L., Kressmann, A., Koski, R. A., Grosschedl, R., Muller, F., Clarkson, S. G., and Birnstiel, M. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2590-2594.

Blunt-IT Repair Kit

Product Code	Pack Size
78420	25 reactions
78421	100 reactions

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