Manual

Version 1.1

Product name: The Blunting Express Kit

Cat #: BLEK-100, BLEK-200, BLEK-300

Description:

The Blunting Express kit can be used to generate blunt-ended DNA fragments for subsequent use in ligation, cloning, cDNA construction, and MCLAB's Topomize Amplicon Library Prep Kit (Cat# TopoA-50A or TOPOA-50B). Our proprietary enzyme mix effectively fills in 5' overhangs and eliminates A-tailed 3' overhangs on the template. This Blunting Express Kit is optimized to provide a rapid workflow with only 2 minutes of incubation. Efficiency is combined with a high performance to streamline your blunt-end objectives.

Features:

Due to our proprietary Enzyme Mix, this kit provides a rapid workflow with a blunting step that requires only two minute incubation. Its high efficiency is combined with a high performance to streamline the blunt end cloning objectives.

Recommended Storage Condition: -20 °C

Kit Contents:

- 10x Buffer
  500mM Potassium Acetate
  200mM Tris-acetate 100mM
  Magnesium Acetate 1mg/ml BSA
  pH 7.9@25°C
- dNTP Mix (25mM)
- Enzyme Mix

Blunting Protocol:

1. Mix the following components gently and proceed directly to next step.

Components	Amount
DNA	up to 1 µg*
10x Buffer	1 µL
dNTP Mix	0.5 µL
Enzyme Mix	1 µL
Water	to 10 µL

2. Incubate the reaction mixture at 72°C for 2 minutes, then place on ice.
3. Purification is recommended before any further reactions (see the protocol below). Purification is not needed for any reactions with MCLAB's AmpliSeq.

   * Recommended DNA template addition: It is recommended that the amount of DNA template added should be between 100ng and 1µg for optimal reaction efficiency. The protocol can be scaled up as necessary. Purification to any PCR product or other such DNA reaction mix is optional.

The MCLAB recommended purification protocol is provided below.

MCLAB purification protocol:

1. Transfer the DNA mix into a 1.5mL tube.
2. Vortex the magnetic beads until well dispersed, then dispense 1.8X volume of the beads into the tube, and mix thoroughly by pipetting up and down.
3. Incubate the tube at room temperature for 5 min.
4. Place the tube on a magnetic stand for 5 min until the liquid is clear, discard the supernatant.
5. With the tube remaining on the magnetic stand wash with 200 µL of freshly made 80% ethanol without disturbing the beads, and allow the tube to sit on the magnetic stand for 30 seconds. Pipet the ethanol out and repeat this step once more.
6. Use a 20 µL pipette to remove the residual ethanol from each tube.
7. Allow the beads to air dry at room temperature (2-5 min). Try to avoid over-drying the beads, which may result in lower recovery of DNA target.
8. Add the resuspension buffer at any chosen volume to the dry beads, mix thoroughly then incubate off the magnetic stand for 2 min.
9. Place on a magnetic stand and wait until the liquid is clear (2-5 min).
10. Transfer the supernatant to a new tube.