

Bst Adapter Fill-In Kit

Technical Specifications



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Ten reactions
 Catalog No. 40031-1.

Store at -20°C.
 For *In Vitro* Research Use Only.
 Not for Drug or Diagnostic use. Not for use in humans or animals.

Product Description	The Bst Adapter Fill-In Kit combines the strand displacement polymerase from <i>Bacillus stearothermophilus</i> (Bst) with an optimized buffer containing nucleotides which eliminates nicks and gaps in sample DNA.
Storage	Enzyme and buffer should be stored at -20°C.
Stability	Enzyme and buffer are stable for 12 months if handled properly.
Activity Determination	One unit catalyzes the incorporation of 10nmol of dNTP into acid-insoluble material in 30 minutes at 65°C in 20 mM Tris-HCl pH 8.8, 10 mM (NH ₄) ₂ SO ₄ , 10 mM KCl, 2 mM MgSO ₄ , 0.1 % Triton X-100, 30 nM M13mp18 ssDNA, 70 nM M13 sequencing primer (-47) 24 mer, 200 μM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [³³ P]dCTP), and 0.1 mg/ml BSA.
Absence of Endonuclease or Nicking Activity	Incubation of 50 U of Bst Adapter Fill-in Enzyme with 1 μg of supercoiled pBR322 DNA for 16 hours at 37°C resulted in no detectable conversion to relaxed or linear forms by agarose gel electrophoresis.
Absence of Exonuclease Activity	Incubation of 50 U of Bst Adapter Fill-in Enzyme with 1 μg of HindIII-cut lambda DNA for 16 hours at 37°C resulted in no smearing of bands on agarose gels. Single-stranded and double-stranded exonuclease activities were tested by incubating 10 μl of enzyme at 220 U/ μl with radiolabeled DNA substrate for one hour at 37°C, resulting in less than 0.1% release of TCA-soluble counts.
Functional Validation	Each lot is functionally validated in construction and sequencing of a genomic DNA library for a 454 Genome Sequencer.
Purity	Enzyme >99% pure by SDS PAGE. No detectable DNA contamination: 5 μl of enzyme at 50 U/ μl of sample was tested for <i>E. coli</i> genomic DNA contamination by PCR amplification with the <i>E. coli</i> 16S ribosomal primers. Absence of an amplified target judged by agarose gel electrophoresis.

Applications:

- Next Generation sequencing library construction.
- cDNA library construction.
- Removal of nicks or gaps in any DNA sequence.

Reference:

Margulies, M. et al. (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437, 376–380.

Warranty

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Reaction Conditions:

In a 1.7 ml tube, add the following reagents in the order indicated:

DNA in H ₂ O	37.0 μ l
5X Fill-In Buffer	10.0 μ l
Fill-In Enzyme	3.0 μ l
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Total volume	50.0 μ l

Mix well and incubate for 20 minutes at 37°C. The reaction can be terminated by heating at 70°C for 15 minutes or using a DNA purification protocol.