Bsm DNA Polymerase, Large Fragment

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

SKU	Contents	Amount	Storage
EP0691	*Bsm* DNA Polymerase, Large Fragment	1600 U, 8 U/µL	-25 °C to -15 °C
	10X *Bsm* Buffer	1.25 mL	

Description

Bsm DNA Polymerase, Large Fragment is a portion of DNA polymerase of *Bacillus smithii*, which catalyzes 5'→3' synthesis of DNA and lacks 5'→3' and 3'→5' exonuclease activities. *Bsm* DNA Polymerase, Large Fragment has a strong strand displacement activity and is active in a wide range of temperatures from 30 °C to 63 °C, with an optimum of activity at 60 °C. *Bsm* DNA Polymerase, Large Fragment is an enzyme with high functional similarity to *Bst* DNA Polymerase, Large Fragment and can replace it in most applications. The enzyme is not suitable for use in PCR.

Applications

- Isothermal DNA amplification by the method of:
  – loop- mediated isothermal amplification (LAMP) (1, 2),
  – whole genome amplification (WGA) (3),
  – ramification amplification (RAM) (4).
- Random- primed DNA labeling
- Labeling by fill-in 5'-overhangs of dsDNA

Source

E.coli cells with a cloned part of *polA* gene from *Bacillus smithii*.

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 min at 60 °C.

Storage Buffer

The enzyme is supplied in: 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.15 % (v/v) Triton X-100 and 50 % (v/v) glycerol.

10X *Bsm* Buffer

200 mM Tris-HCl (pH 8.8 at 25 °C), 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1 % (v/v) Tween 20.

Inactivation

Inactivated by heating at 80 °C for 10 min.

Recommended Protocol for RT-LAMP:

1. To minimize the risk of contamination, thoroughly clean workspace and equipment. Use nuclease-free tubes and filter tips to minimize RNA degradation. Prepare your RNA samples, setup assay and visualization should be performed in separate areas.
2. Always include several negative control reactions (no template control, non-target template control) to ensure amplification specificity.
3. Use purified RNA samples eluted in RNase-free water.
4. Prepare sufficient master mix for the number of reactions plus one extra. Gently vortex and briefly centrifuge all solutions after thawing. Place all components on ice and add them for each 25 µL reaction in order bellow:

Component	Volume	Final Conc.
RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 U/µL stock)	1 µL	1.6 U/µL
10X *Bsm* Buffer	2.5 µL	1X
$MgCl_2$ (25 mM stock)	4 µL	6 mM*
dNTP mix (10 mM stock)	3.5 µL	1.4 mM each
FIP/BIP primers (40 µM stock)	1 µL each	1.6 µM
F3/B3 primers (10 µM stock)	0.5 µL each	0.2 µM
LoopF/B primers (10 µM stock)	1 µL each	0.4 µM
Nuclease-free water	6 µL	
Bsm DNA Polymerase, large fragment (8 U/µL)	1 µL	0.32 U/µL
SuperScript™ IV Reverse Transcriptase (200 U/ µL)	1 µL	8 U/µL
Purified RNA sample	1 µL	variable
Total reaction volume	**25 µL**	

*2 mM included in 10X *Bsm* buffer

5. Place tubes into a pre-heated thermocycler or heat block set to 60 °C for 30 to 60 minutes. To inactivate, incubate reaction mixture at 95 °C for 2 minutes.

Note: optimal temperature range for *Bsm* is 59-62 °C. Total optimal Mg^{2+} concentration is 6-8 mM. Primers can be mixed before and stored to avoid possible pipetting errors.

6. Optional end-point analysis: (a) green fluorescence (SYBR™ Green I stain) or (b) agarose gel electrophoresis:
   a. Add 1 µL SYBR™ Green I stain (10,000X stock) diluted with nuclease-free water 1:10 (v/v) to each tube. Mix by flicking the tubes and spin down. Green color indicates successful amplification, and orange color indicates no amplification.
   b. Dilute 5 µL of samples and controls in 15 µL of nuclease-free water (gently mix and briefly spin down). Transfer 20 µL of ladder and samples into agarose gel wells and run electrophoresis for 15 min. A ladder-like band pattern indicates successful amplification.

Recommended Protocol for LAMP:

1. Prepare master mix for LAMP as above (step 4), but without SuperScript™ IV Reverse Transcriptase (water volume adjusted) and using purified DNA (or cDNA) sample instead of RNA template.
2. Follow to step 5 of the RT-LAMP protocol.

General Guidelines for Primer Design:

Critical aspects of designing LAMP primers:

1. Amplicon length (<300 bp), distance between F2 and B2 primers is 120-160 bp, loop is 40-60 bp (distance between F1 and F2 primers)
2. 45-60 % GC content, avoid single or dinucleotide base repeats, avoid secondary structure regions
3. Primer stability: 3'-end of F2/B2 and F3/B3; 5'-end of F1c/B1c has the free energy of -4 kcal/mol or less
4. Melting temperature (Tm) should be similar across primer pairs (<5 °C difference)

References

1. Tsugunori Notomi, et al., Loop-mediated isothermal amplification of DNA, Nucleic Acids Res., v. 28, No. 12, e63, 2000.
2. Masaki Imai, et al., Rapid diagnosis of H5N1 avian influenza virus infection by newly developed influenza H5 hemagglutinin gene-specific loop-mediated isothermal amplification method, Journal of Virological Methods, 141, 173-180, 2007.
3. Dean, F.B., et al., Comprehensive human genome amplification using multiple displacement amplification, Proc. Natl. Acad. Sci. USA, 99, 5261-5266, 2002.
4. Jizu Yi, et al., Molecular Zipper: a fluorescent probe for real-time isothermal DNA amplification, Nucleic Acids Res., v. 34, No. 11, e81, 2006.

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