Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 2
Revision date: 05/06/2023

1. Identification

Product name	**Bst DNA Polymerase (Exonuclease Minus)** 2000U (8U/uL)
Cat. No	**P0045**

2. Description

Bst DNA Polymerase Exonuclease Minus is a moderately thermostable enzyme from *Bacillus stearothermophilus* genetically optimized for faster amplification and more flexible reaction conditions.
The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium ions and lacks the 5´→3´ exonuclease activity, while retaining the polymerase activity. Enzyme provide strong strand displacement activity. The optimum temperature is from 60-65 °C. Bst becomes heat inactivated at 80 °C.

3. Protein information

Storage buffer	10 mM Tris-HCl (pH 8.0 at 20˚C), 50 mM KCl, 0.1 mM EDTA, 0.1% Tergitol™ TMN, 1 mM DTT and 50% glycerol.
Reaction Buffer (10X)	500 mM Tris-HCl (pH 8.9 at 20˚C), 100 mM $(NH_4)_2SO_4$, 500 mM KCl, 20 mM $MgSO_4$, 1% Tergitol™ TMN.
Biological activity	One unit is defined as the amount of enzyme required to incorporate 10 nmoles of total deoxyribonucleotide into acid-insoluble material in 30 minutes at 65˚C.

4. Storage specifications

Store at -20˚C.

5. Applications

- Nucleic acid amplification methods, including isothermal amplification.
- Whole genome amplification.
- Multiple displacement amplification.
- Sequencing DNA with high GC content and secondary structures.
- Rapid sequencing from nanogram amounts of DNA template.

6. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. Th product was not tested for use in diagnostics or for drug development, nor is it suitable fc administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belie at the date of publication. This information is intended only as a guide and should not be take as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for an damage resulting from handling or from contact with the above product.

Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 2
Revision date: 05/06/2023

Typical LAMP (Loop-mediated isothermal amplification) reaction:

1.- Reactions should be setup on ice. Pipet with sterile filter tips and make the reaction mix in an area separate from DNA preparation or analysis. No-template controls should be included in all amplification sets.

2.- Prepare LAMP Primer Mix with all 4 or 6 (with Loop) primers. 10X Primer Mix should contain: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 4 µM LoopF, 4 µM LoopB in TE or water.

3.- Use a specific detection instrument for isothermal amplification or a real time PCR cycler to run the assays. Real-time detection of the DNA amplification with a fluorescent dye is highly recommended.

4.- Set th e instrument to a constant incubation temperature between 60 to 65°C (depending on the primer annealing temperature). Measure the fluorescence intensity at an interval of 1 minute for up to 30 min. Prepare the reaction mix as shown in the Table in the order listed. During this step the reaction mix tube should always be held on the ice to prevent the background activity of the enzyme.

Component	Volume (µL)	Final Conc
10X Reaction Buffer	2.5 µl	1X (does not contain MgSO4)
MgSO4 (100 mM)	1.5 µl	8 mM total
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
10X LAMP Primer Mix	2.5 µl	1X
Bst DNA Polymerase (8 U/µl)	1 µl	0.32 U/µl
DNA Sample	variable	> 10 copies or more
Nuclease-free Water	to 25 µl	

1. If reaction optimization is needed:
   •Change Mg2+ concentration in a range of 4—10 mM.
   •Choose a different enzyme concentration (0.040—0.4 U/µl).
   •Set other reaction temperature (50 to 68°C).
   •Extend the reaction time. (Prelonged incubation may cause amplification of unspecifically annealed primers or primer dimer formations).

2. For difficult DNA templates pre-warm the reaction mix witout enzyme at 92°C for 1 min. Cool down to room temperature, then add the Opti Bst DNA Polymerase, mix well, centrifuge and place tubes into a pre-heated thermocycler or heat block.

3. If amplification in no-template controls occurs the following points should be reviewed.
   •Replace reagent stocks and pre-mixes with new components.
   •Use separate preparation area and equipment if post-reaction processing is necessary
   •Avoid opening reaction tubes after amplification.
   •Use dNTPs with 50% inclusion of dUTP mixed with dTTP and 0.2 U of Thermolabile Uracil-N-glycosylase (E1251) per reaction.