DATA SHEET

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Bst 2.0 Polymerase

Bst polymerase optimized for Loop-Mediated Isothermal DNA Amplification (LAMP)

Cat. Nº.	Amount
☐ POL-133XS	500 units
☐ POL-133S	2.000 units
☐ POL-133L	10.000 units

Concentration:
8 units/µL

Shipping:
Shipped on blue ice

Storage Conditions:
Store at -20 °C

For *in vitro* use only!

Additional Storage Conditions:
Avoid freeze/thaw cycles

Shelf Life:
12 months

Description:
Bst 2.0 Polymerase is a genetically enhanced Bst polymerase of the next generation. The polymerase is the ideal choice for robust amplification of DNA at constant temperature (60 to 65 °C). The enzyme shows high strand displacement activity and generates an amplification factor of up to 10^9 which is comparable to approx. 30 cycles in a PCR assay. The polymerase allows detection of a target gene within 30-60 minutes.

Kit contents:

Bst 2.0 Polymerase (blue cap)
8 units/µl Bst DNA Polymerase in 10 mM Tris-HCl, 50 mM KCl, 50 % (v/v) Glycerol, pH 7.5 (25 °C) and stabilizers.

Isothermal Buffer (red cap) - 10x conc.
200 mM Tris-HCl pH 8.8, KCl, $(NH_4)^2SO_4$, 20 mM $MgSO_4$ and detergents.

$MgSO_4$ Stock Solution (yellow cap)
25 mM $MgSO_4$.

Detection
Although some methods have been developed to visualize DNA amplification by basic equipment or even the naked eye (increase of turbidity, color change of added dyes, hybridization to gold-bound ss-DNA) in general real-time detection of the DNA amplification by a fluorescent DNA-intercalator dye is recommended. Addition of EvaGreen Fluorescent DNA Stain (#PCK-122, not provided) to the assay allows a sensitive measurement of the increasing amount of DNA without influence on the reaction.

Assay design
Isothermal amplification is an extremely sensitive detection method and care should be taken to avoid contamination of set-up areas and equipment with DNA of previous reactions. A common problem is amplification in no-template controls due to:

1. carry-over contamination or
2. amplification of unspecifically annealed primers or primer dimer formations.

As sensitivity and non-template amplification of in-silico designed primers may vary, the evaluation of 2-4 real primer sets before choosing a final set is recommended.

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Assay set-up

Depending on the detection method and machine a reaction volume of 20-50 µl is recommended for most applications. Pipet with sterile filter tips and perform the set-up in an area separate from DNA preparation or analysis. No-template controls should be included in all amplifications. First, prepare a 10x conc. primer pre-mix. A 10x primer mix should contain: 16 µl FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 4 µM LoopF, 4 µM Loop B in TE buffer or water. Second, set-up the isothermal amplification assay:

Components	25 µl reaction	[final]
Isothermal Buffer (10x)	2,5 µl	1x
$MgSO_4$ (25 mM)	4 - 6 µl	6 - 8 mM
dNTP (Mix 10 mM)	3,5 µl	1,4 mM
Primer Mix (10x)	1,25 - 2,5 µl	0,5 - 1x
Bst 2.0 (8 U/µl)*	1 µl	0,32 units/µl
EvaGreen DNA Stain 50x	0,5 µl	1 x
Template DNA	x µl	1 pg - 10 ng
PCR Grade water	fill up to 25 µl	--

* May vary depending on the application.

- Use a specific detection instrument for isothermal amplification or a real-time PCR cycler to run the assays
- Set the 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 min for up to 60 min

Optimization of $MgSO_4$ concentration:

Isothermal buffer provides a final Mg^{+2} concentration of 2 mM. However, most LAMP reactions requires a final Mg^{+2} concentration between 6 - 8 mM. To optimize Mg^{+2}, add 25 mM of $MgSO_4$ stock solution as shown in the table.

final $MgSO_4$ conc.	25 µL reaction
6 mM	4 µl
7 mM	5 µl
8 mM	6 µl

Trouble shouting

If amplification in no-template controls occurs the following points should be reviewed.

Cross contamination from environments

- Clean equipment and areas with "DNA Away" solution
- Replace reagent stocks and pre-mixes with new components
- Stop reactions at an earlier point of time before non-template amplification occur

Carry-over contamination from previous reaction products

- Avoid opening reaction vessels after amplification
- Use separate preparation area and equipment if post-reaction processing is necessary
- Add UNG Heat Labile (#ENZ-126, not provided) in your reaction.

Non-template amplification from primers

- Increase incubation temperature stepwise by 1-2 °C
- Design a new set of primers for the target sequence