DATA SHEET

cellco
Biotecnologia ao seu alcance

Bst 2.0 Turbo Polymerase

Bst polymerase for isothermal DNA amplification
Isothermal Amplification

Cat. Nº.	Amount
■ POL-134XS	500 units
□ POL-134S	2.000 units
□ POL-134L	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:
Saphir Bst2.0 Turbo Polymerase is a genetically enhanced Bst2.0 polymerase of the next generation. The polymerase is the ideal choice for ultra-fast and 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 is 2-3 x faster compared to convencional Bst Polymerase and allows detection of a target gene within 10-30 minutes.

Kit contents:

Bst2.0 Turbo Polymerase (blue cap)
8 units/µl Bst DNA Polymerase in 10 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1 % Triton X-100, 50 % (v/v) Glycerol, pH 7.5 (25 °C).

Bst2.0 Turbo Buffer (red cap) - 10x conc.
200 mM Tris-HCl pH 8.8, 1 M KCl, 100 mM (NH4)2SO4, 60 mM $MgSO_4$, stabilizers 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) 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.

DATA SHEET

cellco
Biotecnologia ao seu alcance

Bst 2.0 Turbo Polymerase

Bst polymerase for isothermal DNA amplification
Isothermal Amplification

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	50 µl rxn	[final]
Bst 2.0 Turbo Buffer 10x	5	1x
$MgSO_4$ Stock Solution	0-4 µl	0-2 mM
dNTP (Mix 10 mM)	7 µl	1,4 µM
Primer Mix	2 µl	1x
Bst 2.0 Turbo Polymerase	2 µl	0,32 units/µl
EvaGreen DNA Stain	0,65 µl	1,3 mM
Template DNA	x µl	<500 ng/assay
PCR Grade water	fill up to 50 µl	--

- 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 30 min

Optimization of $MgSO_4$ concentration:

A final Mg^{2+} concentration of 6.0 mM (as contained in the reaction buffer) is optimal for most primer-template combinations. However, if an individual Mg^{2+} optimization is essential add 25 mM $MgSO_4$ stock solution as shown in the table below.

final $MgSO_4$ conc.	50 µL rxn
6 mM	-
7 mM	2 µl
8 mM	4 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

Non-template amplification from primers

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