DATA SHEET

Jena Bioscience

Saphir Bst Polymerase

Bst polymerase for isothermal DNA amplification
Isothermal Amplification

Cat. No.	Amount
PCR-389S	2.000 Units
PCR-389L	5 x 2.000 Units

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Concentration: 8 units/µl

Description:
Saphir Bst Polymerase is a genetically improved Bst polymerase for rapid and specific 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. This allows detection of a target gene within 10-30 minutes.

Content:
Saphir Bst Polymerase
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.

Saphir Bst Buffer
10 x conc. complete reaction buffer containing 200 mM Tris-HCl pH 8.8, KCl, $(NH_4)_2SO_4$, 20 mM $MgSO_4$ and detergents.

$MgSO_4$ Stock Solution
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 a Fluorescent DNA Stain 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. Second, set-up the isothermal amplification assay:

component	stock conc.	final conc.	20 µl	50 µl
Saphir Bst Buffer	10x	1x	2 µl	5 µl
$MgSO_4$ Stock Solution *	25 mM	0-2 mM	0-1.6 µl	0-4 µl
dNTP Mix	10 mM	1.4 mM	2.8 µl	7 µl
Primer Mix	10x	1x	2 µl	5 µl
Saphir Bst Poly-merase	8 units/µl	0.32 units/µl	0.8 µl	2 µl
EvaGreen DNA Stain (#PCR-379)	100 µM	1.3 µM	0.26 µl	0.65 µl
Template DNA		<500 ng/assay	x µl	x µl
PCR-grade Water			fill up to 20 µl	fill up to 50 µl

* optional, please refer to the table below

- 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 already 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.	20 µl final assay volume	50 µl final assay volume
6 mM	- µl	- µl
7 mM	0.8 µl	2.0 µl
8 mM	1.6 µl	4.0 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

Related Products:
dNTP Mix / 10 mM, #NU-1006
dNTP Mix / 25 mM, #NU-1023