DATA SHEET

Saphir Bst 2.0 Polymerase

Bst polymerase for isothermal DNA amplification
Isothermal Amplification

Cat. No.	Amount
PCR-389S	2.000 Units
PCR-389L	10.000 Units

For *in vitro* use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Concentration: 8 units/µl

Description:
Saphir Bst 2.0 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 2.0 Polymerase (red cap)
8 units/µl Bst DNA Polymerase in 10 mM Tris-HCl, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1 % Triton X-100, 50 % (v/v) Glycerol, pH 7.5 (25 °C)

Saphir Bst 2.0 Buffer (blue cap)
10 x conc. complete reaction buffer containing 200 mM Tris-HCl pH 8.8, 500 mM KCl, 100 mM $(NH_4)_2SO_4$, 60 mM $MgSO_4$, stabilizers and detergents

$MgCl_2$ Stock Solution (yellow cap)
25 mM $MgCl_2$

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 SybrGreen or EvaGreen Fluorescent DNA Stain (#PCR-378, #PCR-379) 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 2.0 Buffer	10x	1x	2 µl	5 µl
$MgCl_2$ Stock Solution	25 mM	0-2 mM	0-1.6 µl	0-4 µl
dNTP Mix	10 mM	400 µM	0.8 µl	2 µl
Primer Mix	10x	1x	2 µl	5 µl
Saphir Bst 2.0 Poly-merase	8 units/µl	0.32 units/µl	0.8 µl	2 µl
EvaGreen DNA Stain	100 µM	2.5 mM	0.5 µl	1.25 µl
Template DNA		<500 ng/assay	x µl	x µl
PCR-grade Water			fill up to 20 µl	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 $MgCl_2$ 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 $MgCl_2$ stock solution (#PCR-266) as shown in the table below.

final $MgCl_2$ conc.	20 µl final assay volume	50 µl final assay volume
6 mM	-	-
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
- Use a master mix with UNG and dUTP instead of dTTP or add both components separately to the assay to prevent carry-over contamination

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:
SybrGreen DNA Stain, #PCR-378
EvaGreen DNA Stain, #PCR-379
$MgCl_2$ Stock Solution, #PCR-266
dNTP Mix / 10 mM, #NU-1006
dNTP Mix / 25 mM, #NU-1023