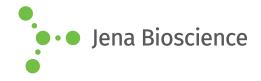
# **DATA SHEET**





# 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 109 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/ $\mu$ l Bst DNA Polymerase in 10 mM Tris-HCl, 50 mM KCl, 50 % ( $\nu$ / $\nu$ ) 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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM MgSO<sub>4</sub> and detergents.

## MgSO<sub>4</sub> Stock Solution

25 mM MgSO<sub>4</sub>.

### 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.

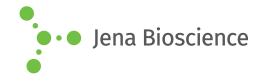
### **Assay design**

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.

# **DATA SHEET**





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### Assay set-up

Depending on the detection method and machine a reaction volume of 20-50  $\mu l$  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 <sub>4</sub> 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 μΜ	1.3 μΜ	0.26 μl	0.65 μl
Template DNA		<500 ng/assay	xμl	xμl
PCR- grade Water			fill up to 20 µl	fill up to 50 µl

<sup>\*</sup> 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<sub>4</sub> concentration:

A final  $\mathrm{Mg}^{2^+}$  concentration of 6.0 mM (as already contained in the reaction buffer) is optimal for most primer-template combinations. However, if an individual  $\mathrm{Mg}^{2^+}$  optimization is essential add 25 mM  $\mathrm{MgSO}_4$  stock solution as shown in the table below.

final MgSO <sub>4</sub> 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