

Saphir Bst2.0 Turbo Polymerase

Cat#: orb611228 (Product Manual)

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/ μ l

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 109 which is comparable to approx. 30 cycles in a PCR assay. The polymerase is 2-3 x faster compared to Saphir Bst2.0 Polymerase and allows detection of a target gene within 5-10 minutes.

Content:

Saphir Bst2.0 Turbo Polymerase

8 units/ μ l 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)

Saphir Bst2.0 Turbo Buffer

10 x conc. complete reaction buffer containing 200 mM Tris-HCl pH 8.8, 1 M KCl, 100 mM (NH₄)₂SO₄, 60 mM MgSO₄, stabilizers and detergents

MgCl₂ Stock Solution

25 mM MgCl₂

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 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.

Assay set-up

Depending on the detection method and machine a reaction volume of 20-50 μ 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 μ l	50 μ l
Saphir Bst2.0 Turbo Buffer	10x	1x	2 μ l	5 μ l
MgCl ₂ Stock Solution	25 mM	0-2 mM	0-1.6 μ l	0-4 μ l
dNTP Mix	10 mM	1.4 μ M	2.8 μ l	7 μ l
Primer Mix	10x	1x	2 μ l	5 μ l
Saphir Bst2.0 Turbo Polymerase	8 units/ μ l	0.32 units/ μ l	0.8 μ l	2 μ l
EvaGreen DNA Stain	100 μ M	1.3 mM	0.26 μ l	0.65 μ l
Template DNA		<500 ng/assay	x μ l	x μ l
PCR-grade Water			fill up to 20 μ l	fill up to 50 μ l

- 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 20 min.

Optimization of MgCl₂ concentration:

A final Mg²⁺ concentration of 6.0 mM (as contained in the reaction buffer) is optimal for most primer - template combinations. However, if an individual Mg²⁺ optimization is essential add 25 mM MgCl₂ stock solution 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 shooting

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