



Saphir Bst Turbo Polymerase

Bst polymerase for extremely fast isothermal DNA amplification
Isothermal Amplification

Cat. No.	Amount
PCR-390S	2.000 Units
PCR-390L	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/ μ l

Description:

Saphir Bst Turbo Polymerase is a genetically enhanced Bst 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 Saphir Bst Polymerase (#PCR-389) and allows detection of a target gene within 5-10 minutes.

Content:

Saphir Bst 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 Bst Turbo Buffer

10 x conc. complete reaction buffer containing 200 mM Tris-HCl pH 8.8, 1 M KCl, 100 mM $(\text{NH}_4)_2\text{SO}_4$, 60 mM MgSO_4 , stabilizers 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 Turbo 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 Turbo Polymerase	8 units/µl	0.32 units/µl	0.8 µl	2 µl
EvaGreen DNA Stain	100 µM	1.3 mM	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 20 min.

Optimization of MgSO₄ concentration:

A final Mg²⁺ concentration of 6.0 mM (as already contained in the reaction buffer) is optimal for most primer-template combinations. However, if an individual Mg²⁺ optimization is essential add 25 mM MgSO₄ stock solution (#PCR-266) as shown in the table below.

final MgSO ₄ 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

Related Products:

MgCl₂ Stock Solution, #PCR-266
dNTP Mix / 10 mM, #NU-1006
dNTP Mix / 25 mM, #NU-1023