DATA SHEET

Jena Bioscience

Saphir Bst Turbo GreenMaster

Master mix for isothermal DNA amplification with SYBR® Green

Isothermal Amplification

Cat. No.	Amount
PCR-393S	2 x 1,25 ml
PCR-393L	10 x 1,25 ml

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: store dark

Short term storage (up to 3 months) at 4 °C possible.

Shelf Life: 12 months

Form: liquid

Concentration: 2x conc.

Spectroscopic Properties: λ_{exc} 495 nm (SYBR® Green bound to DNA), λ_{em} 520 nm (SYBR® Green bound to DNA)

Description:
Saphir Bst Turbo GreenMaster is a complete 2x conc. master mix for isothermal amplification of DNA. The mix is based on a genetically enhanced Bst polymerase of the next generation. The mixe 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 2-3x faster compared to Saphir Bst Polymerase (#PCR-389/#PCR-387) and allows detection of a target gene within 5-10 minutes.

Content:
Saphir Bst Turbo GreenMaster
Saphier Bst Turbo Polymerase, dNTPs, reaction buffer, glycerol, SYBR® Green DNA intercalator dye, stabilizers

PCR-grade water

Detection
The mix contains the fluorescent DNA stain SYBR® Green that intercalates into DNA during the amplification process and allows the direct quantification of target DNA by fluorescence detection (analogous to real-time PCR).
The mix can be combined with ROX reference dye (#PCR-351) to allow a signal normalization in real-time PCR instruments that are compatible with the evaluation of the ROX signal.

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 problem may be amplification in no-template controls due to carry-over contamination or amplification of unspecifically annealed primers or primer dimer formations.

Primer design
Typically, 4 different primers are used to identify 6 distinct DNA regions allowing the specific amplification of a target gene. An additional pair of primers further accelerates the amplification allowing to cut down the total detection time to 5-10 min.
The manual design of primers may be challenging due to the complex reaction sequence. To simplify the design process the use of a primer design software is recommended.
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
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 Green-Master	2x	1x	10 µl	25 µl
Primer Mix	10x	1x	2 µl	5 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.

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:
Saphir Bst Turbo Polymerase, #PCR-390
SYBR® Green DNA Stain, #PCR-378
$MgCl_2$ Solution, #PCR-266
dNTP Mix / 10 mM, #NU-1006
dNTP Mix / 25 mM, #NU-1023