DATA SHEET

Jena Bioscience

Saphir Bst GreenMaster highROX

Master mix for isothermal DNA amplification with EvaGreen and ROX
Isothermal Amplification

Cat. No.	Amount
PCR-388S	2 x 1,25 ml
PCR-388L	10 x 1,25 ml

For *in vitro* use only!

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: EvaGreen bound to DNA: λ_{exc} 500 nm, λ_{em} 530 nm
ROX passive reference dye: λ_{exc} 576 nm, λ_{em} 601 nm

Description:
Saphir Bst GreenMaster highROX is a complete 2x conc. master mix for isothermal amplification of DNA. The mix is based on a genetically optimized Bst polymerase that allows 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. LAMP technique allows detection of a target gene within 10 - 30 minutes.

Content:
Saphir Bst GreenMaster highROX (purple cap)
Saphier Bst Polymerase, dNTPs, reaction buffer, glycerol, EvaGreen DNA intercalator dye, ROX passive reference dye, stabilizers

PCR-grade water

Detection
The mix contains the fluorescent DNA stain EvaGreen® 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 contains 500 nM ROX passive reference dye in the final assay. The dye does not take part in the PCR reaction but allows to normalize for non-PCR related signal variation and provides a baseline in multiplex reactions.

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 10-20 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 Green-Master highROX	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 Polymerase, #PCR-389
EvaGreen DNA Stain, #PCR-379
$MgCl_2$ Stock Solution, #PCR-266
dNTP Mix / 10 mM, #NU-1006
dNTP Mix / 25 mM, #NU-1023