



Saphir LAMP Turbo GreenMaster Lyophilisate

Lyophilisate for extremely fast isothermal DNA amplification with green-fluorescent DNA stain

Cat. No.	Amount
PCR-395S	192 reactions (2x 96-well plates)
PCR-395L	960 reactions (10x 96-well plates)

For general laboratory use.

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Additional Storage Conditions: Store in an aluminium-coated bag or on a dry place.

Lyophilisates may hydrate at humidity levels >70 % when sealing is opened.

Shelf Life: 6 months in sealed packaging

Description:

Saphir LAMP Turbo GreenMaster Lyophilisate is designed for isothermal amplification of DNA. The mix is based on a genetically enhanced Bst polymerase of the next generation. The mix 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 2-3x faster compared to Saphir Bst Polymerase (#PCR-389/#PCR-388/#PCR-387) and allows detection of a target gene within 5-10 minutes.

Content:

Saphir LAMP Turbo GreenMaster Lyophilisate

Saphir Bst Turbo Polymerase, dNTPs, reaction buffer, Green DNA intercalator dye, additives and stabilizers

PCR grade water

Handling

The lyophilisates are provided in low-profile (0.1 ml) 96-well plates with optically clear caps, whereby each well contains reaction mix for a final volume of 20 µl. The plates can be easily divided into 8-well strips and further segmented by cutting, allowing compatibility with a variety of PCR cyclers.

The lyophilisate combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations.

Each vial contains all components (except primers and template) required for a 20 µl LAMP assay.

To perform the Assay, only fill up the vials with a primer mix and add DNA template.

The lyophilisate can also be used with ROX reference dye in PCR instruments that are compatible with the evaluation of the ROX signal. In this case, the ROX dye (#PCR-351) should be added as 1x concentration to the PCR 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 problem may be amplification in no-template controls due to carry-over contamination or amplification of unspecifically annealed primers or primer dimer formations.



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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.

Assay set-up

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.	Volumne 1x 20 µl Assay
Primer Mix	10x	1x	2 µl
Template DNA		<500 ng/assay	x µl
PCR-grade Water			fill up to 20 µl

Dispensing the master mix

Vortex the primer/probe mix thoroughly to assure homogeneity. Dispense 20 µl to each PCR tube or well of the plate.

- Use a specific detection instrument for isothermal amplification or a real-time PCR cyler 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

Saphir LAMP Turbo GreenMaster, #PCR-393

Saphir LAMP Turbo GreenMaster high ROX, #PCR-394