DATA SHEET

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Bst Turbo GreenMaster Lyophilisate

Lyophilisate for isothermal DNA amplification with green-fluorescent DNA stain

Cat. Nº.	Amount
☐ POL-151S	192 reactions x 20 µL
☐ POL-151L	960 reactions x 20 µL

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.

For in vitro use only!

Shelf Life:
6 months in sealed packaging

Description:
Bst 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 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 109 which is comparable to approx. 30 cycles in a PCR assay. The polymerase is 2-3x faster compared to Bst Polymerase (#POL-133) and allows detection of a target gene within 5-10 minutes.

Content:

Bst Turbo GreenMaster Lyophilisate

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

PCR-grade water

Handling

Bst Turbo GreenMaster Lyophilisate is delivered is delivered in 8-tube strips or 96-well plates preloaded with a complete master mix in a dry, room temperature stable format. 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.

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.	Vol. for 20µL mix
Primer Mix	10 x	1 x	2 µL
Template DNA		<500 ng/assay	x µL
PCR-grade water	-	-	Fill up to 20 µL

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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 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