HybriDetect LAMP Hot Start Polymerase (REF: HLHSP 1)

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REF	SIZE	VOLUME	COMPONENT	COMPONENT COMPOSITION
HLP 1	8000 u	8000 u	HybriDetect LAMP Hot Start Polymerase (8 u/µl)	Glycerol-free formulation of the recombinant Bst DNA Polymerase (large fragment) in storage buffer. Excellent for both common and lyo-ready workflows. HybriDetect LAMP Buffer (10x) contains optimal concentration of dNTPs and magnesium sulphate as well as stabilizers. The HybriDetect LAMP Enhancer accelerates the reaction.
		2 x 1.25 ml	HybriDetect LAMP Buffer (10x)	
		3 x 1.7 ml	HybriDetect LAMP Enhancer (5x)	

Storage: In the dark at -20°C *Avoid multiple freezing-thawing, aliquot the enzyme into 5-10 sterile tubes.* *not for diagnostic purposes*

APPLICATIONS

- Isothermal DNA amplification at elevated temperature
- Real-time detection of DNA amplification
- MDA – multiple displacement amplification
- LAMP - loop-mediated isothermal amplification
- WGA - whole genome amplification
- RAM - ramification & RPA - recombinase polymerase ampl.
- Directly compatible with our universal HybriDetect lateral flow strips

BENEFITS

- The enzyme is blocked at ambient temperature due to molecular inhibition based hot-start technology, what is excellent for ambient reaction setup
- Active at high temperatures in a range of 55-70°C
- Fast DNA amplification at constant temperature
- Ideal for complex templates and crude samples
- Ensures <3 molecules LOD (limit of detection)

PRODUCT DETAILS

The HybriDetect LAMP Hot Start Polymerase is a recombinant protein, representing a large fragment of the *B. stearothermophilus* DNA Polymerase expressed in *E. coli* cells. The activity of the enzyme is blocked at ambient temperature due to molecular inhibition based hot-start technology, and the polymerase is activated only at 45-50°C, which reduces non-specific amplification, primer dimer formation. This robust polymerase with a strong strand displacement activity and high temperature tolerance ensures high amplification yield at constant temperature when working with impure or low-copy number targets as well as with complex templates. The HybriDetect LAMP Buffer (10x) includes optimal concentrations of magnesium and dNTPs, what minimizes pipetting steps. This buffer system together with a supplied HybriDetect LAMP Enhancer (5x), detects <3 DNA targets in a short time. The kit is directly compatible with our HybriDetect lateral flow test kits.

PERFORMANCE

Technical characteristics of Bst DNA Polymerase (large fragment):

- The polymerase is blocked at ambient temperature and activated at temperatures above 45°C.
- Strong 5′ - 3′ strand displacement activity
- 5′ - 3′ polymerase activity
- No 5′ - 3′ exonuclease activity
- No 3′ - 5′ exonuclease (proofreading) activity
- Minor reverse transcriptase activity (for RNA, RTase is needed)
- The optimal amplification temperature is 65°C.
- The working temperature range is 55 - 70°C.
- The optimal reaction time is 20 minutes depending on the buffer system
- If needed, the reaction can be extended to 30 - 60 minutes
- The enzyme is inactivated in 10 minutes at 80°C.

ISOTHERMAL AMPLIFICATION PROTOCOL EXAMPLE

- Take typical measures to prevent contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive controls in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Ambient temperature assembly can be performed with the HS Mix, as the enzyme becomes activated only above 45°C.
- Perform the reaction at 65°C. If needed, optimize the reaction temperature between 55 - 70°C for each template/primers system. Complex templates may require higher temperature.
- The suggested reaction time is 20 - 30 minutes. For some low copy number targets 30 - 60 minutes might be required.
- Design primers with predicted melting temperature of about 60°C.
- For evaluation with our lateral flow kits use labelled primer pairs.
- Store reactions for short time on ice, for long time at -20°C.
- High yield amplification product may increase the viscosity of the solution, mix well and dilute if needed for downstream applications.

Prepare primer mix (10x) in water or TE Buffer, for example, for LAMP: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 8 µM LoopF, 8 µM LoopB.

Prepare a 25 µl reaction		
- HybriDetect LAMP Buffer (10x)		2.5 µl
- HybriDetect LAMP Enhancer (5x)		5 µl
- Primer Mix (10x)		2.5 µl
- Template DNA		1 µl
- PCR grade water		to 24 µl
- HybriDetect LAMP Hot Start Polymerase (8 u/µl)		1.0 µl
Mix gently, avoid bubbles.		
Incubate in a thermostat or qPCR instrument:		
- Amplification	65°C	20-30 min
- Inactivation (optional)	80°C	10 min
Lateral Flow Evaluation		
- add 2-10 µl LAMP to the test strip		
- apply 40-120 µl running buffer		
- incubate for 5 min		

All HybriDetect LAMP Kits are directly compatible with our universal HybriDetect lateral flow kits (MGHD 1, MGHD2 1 and MGHC 1) for easy and fast evaluation.

Note: Laboratory Chemical - not for diagnostic purposes. The use of this product in certain applications may be covered by patents. The user has to analyse all applicable Limited Use Label Licenses and may need licensing for certain cases.

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