HybriDetect LAMP Kit (REF: HLK 1)

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REF	SIZE	VOLUME	COMPONENT	COMPONENT COMPOSITION
HLK 1	100 reactions	1.25 ml 0.125 ml 1 ml	HybriDetect LAMP Mix (2x) HybriDetect LAMP Fluorescence Dye (20x) HybriDetect PCR grade water	1x HybriDetect LAMP Mix includes recombinant Bst DNA Polymerase (large fragment), 3 mM $MgSO_4$, 1.6 mM dNTPs, enhancers, stabilizers. HybriDetect LAMP Fluorescence Dye is not needed if LAMP reaction is evaluated with lateral flow. HybriDetect LAMP Fluorescence Dye is to be used if the reaction is run in qPCR cyclers and real-time detection is performed in FAM channel.

Storage: In the dark at -20°C

not for diagnostic purposes

APPLICATIONS

- Isothermal DNA amplification at elevated temperature
- Real-time detection of DNA amplification
- LAMP - loop-mediated isothermal amplification
- WGA - whole genome amplification
- RAM - ramification amplification
- Directly compatible with our universal HybriDetect lateral flow strips

BENEFITS

- Efficient 20 minutes DNA amplification at constant 55 - 70°C temperature
- Supplied with water and a dye for fast real-time detection
- Bst DNA Polymerase with strong strand displacement activity
- Robust on complex templates and crude samples
- Low-copy (<5 molecules) targets detection

PRODUCT DETAILS

HybriDetect LAMP Kit enables detection of as little as 5 target DNA molecules in a short time of 20 minutes even without the use of a thermal cycler. The Kit includes a 2x master mix with optimized high-performance buffer, dNTPs and a recombinant Bst Polymerase large fragment having strong 5′ - 3′ strand displacement activity and efficient 5′ - 3′ polymerase activity working at 55-70°C. The Polymerase has neither 5′ - 3′ nor 3′ - 5′ exonuclease activity and retains only minor reverse transcription activity. The kit includes PCR water; only templates and primers have to be supplied by the user. The kit is directly compatible with our HybriDetect lateral flow test kits. Fluorescence Dye (20x) is included for an optional use, for a real-time detection in FAM channel on any qPCR cycler.
HybriDetect LAMP Kit is a tool of choice for such applications like LAMP, WGA, RAM with an additional advantage of higher temperature reactions, what makes amplification of complex and GC-rich templates more efficient.

PERFORMANCE

Technical characteristics of Bst DNA Polymerase (large fragment):

- 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, if needed, the reaction can be prolonged to 30 - 60 minutes.
- The enzyme is inactivated in 10 minutes at 80°C.
- The Fluorescence Dye has the excitation max. at 482 nm and emission max. at 512 nm.

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.
- 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.
- Fluorescence Dye (20x) shall be used only when performing the reaction in a real-time cycler. Detection is performed in FAM channel, acquiring data each 15 seconds.
- For evaluation with lateral flow no Fluorescence Dye (20x) is needed.
- 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 Mix (2x)		12.5 µl
- Optional: Fluorescence Dye (20x)		1.25 µl
- Primer Mix (10x)		2.5 µl
- Template DNA		1.0 µl
- PCR grade water		to 25 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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