Check the product label for actual catalog number, lot and expiry date.

ALLin™ HS Isothermal DNA Amplification Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IDK0201	100 r of 25 µl	1.25 ml - ALLin™ HS Isothermal Amplification Mix, 2X 0.125 ml - Quantitative Fluorescent Dye, 20X 1 ml - PCR Water	The 1X HS Isothermal Amplification Mix includes a Hot Start Bst Polymerase (large fragment), 3 mM $MgSO_4$, 1.6 mM dNTPs, enhancers, stabilizers. Quantitative Fluorescent Dye is to be used if the reaction is run in qPCR cyclers and real-time detection is performed in FAM channel.
IDK0205	500 r of 25 µl	5 x 1.25 ml - ALLin™ HS Isothermal Amplification Mix, 2X 5 x 0.125 ml - Quantitative Fluorescent Dye, 20X 5 x 1 ml - PCR Water	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- Isothermal DNA amplification at elevated temperature
- Real-time detection of DNA amplification
- LAMP - loop-mediated isothermal amplification
- WGA - whole genome amplification
- RAM - ramification & RPA - recombinase polymerase ampl.

BENEFITS

- Hot Start Bst for an ambient temperature setup and minimized background
- Efficient 20 minutes DNA amplification at constant 55-70°C temperature
- ALLin™ format, supplied with water and a dye for fast real-time detection
- Hot Start Bst DNA Polymerase with strong strand displacement activity
- Robust on complex templates and crude samples
- Low-copy (<3 molecules) targets detection

PRODUCT DETAILS

ALLin™ HS Isothermal DNA Amplification Kit enables detection of as little as 3 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 Hot Start Bst Polymerase having strong 5′ - 3′ strand displacement activity and efficient 5′ - 3′ polymerase activity working at 55 - 70°C. 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 what reduces non-specific amplification, primer dimer formation and is therefore excellent for an ambient temperature reaction setup. 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. Quantitative Fluorescent Dye is included for an optional use, for a real-time detection in FAM channel on any qPCR cycler.

ALLin™ HS Isothermal DNA Amplification Kit is a tool of choice for such applications like LAMP, WGA, RAM with an additional advantage of ambient temperature set up and higher temperature reactions, what makes amplification of complex and GC-rich templates more efficient.

PERFORMANCE

Technical characteristics of Hot Start Bst 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, use ALLin™ Isothermal 1Step RNA Amplification Kit (#IRK0101) which includes RTase)
- Optimal amplification temperature is 65°C.
- Working temperature range is 55 - 70°C.
- Optimal reaction time is 20 minutes, if needed, the reaction can be extended to 30 - 60 minutes
- The enzyme is inactivated in 10 minutes at 80°C.
- Quantitative Fluorescent Dye has the excitation max. at 482 nm and emission max. at 512 nm.

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.

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.
- Suggested reaction time is 20 - 30 minutes. For some low copy number targets 30 - 60 minutes might be required.
- Quantitative Fluorescent 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.
- Design primers with predicted melting temperature of about 60°C.
- Prepare 10X primer mix 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.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 25 µl reaction:

Quantitative Fluorescent Dye, 20X

µl

(variable, depends on application)

✓ Mix gently, avoid bubbles.

✓ Place into the thermostat or qPCR instrument to incubate:

55 - 70°C, time between 20 - 60 min

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.

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