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

ALLin™ Isothermal 1Step RNA Amplification Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
IRK0101	100 r of 25 µl	1.25 ml - ALLin™ Isothermal Amplification Mix, 2X 0.125 ml - Quantitative Fluorescent Dye, 20X 0.2 ml - RT5 Mix 1 ml - PCR Water	1X amplification mix includes recombinant Bst DNA Polymerase (large fragment), 3 mM $MgSO_4$, 1.6 mM dNTPs, enhancers, stabilizers. RT5 Mix includes thermostable reverse transcriptase (modified MMuLV) and a thermostable RNase inhibitor. Quantitative Fluorescent Dye, 20X is to be used if the reaction is run in qPCR cyclers and real-time detection is performed in FAM channel.
IRK0105	500 r of 25 µl	5 x 1.25 ml - ALLin™ Isothermal Amplification Mix, 2X 5 x 0.125 ml - Quantitative Fluorescent Dye, 20X 5 x 0.2 ml - RT5 Mix 5 x 1 ml - PCR Water	

Storage *In the dark at -20°C.*

APPLICATIONS

- Virus RNA detection, SARS-CoV-2 detection
- Isothermal one step RNA amplification at elevated temperature
- RT-LAMP – reverse transcription loop-mediated isothermal amplification
- Molecular diagnostics

BENEFITS

- Efficient 30 minutes 1 Step RNA amplification at 55-70°C temperature
- ALLin™ format, supplied with water and a dye for fast real-time detection
- Bst DNA polymerase with strong strand displacement activity
- Thermostable reverse transcriptase blended with RNase inhibitor
- Robust on complex RNA templates and crude samples
- Low-copy (<5 molecules) targets detection

PRODUCT DETAILS

ALLin™ Isothermal 1Step RNA Amplification Kit enables detection of < 5 target RNA molecules in a short time of 30 minutes without the use of PCR cycler. An RT5 Mix, the blend of a thermostable reverse transcriptase and highly efficient RNase Inhibitor is included in the kit to initially synthesize cDNA from RNA templates. The cDNA is amplified by Bst polymerase. 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 also includes PCR water; only templates and primers have to be supplied by the user. Quantitative Fluorescent Dye, 20X is included for an optional use, for a real-time detection in FAM channel on any qPCR cycler. ALLin™ Isothermal 1Step RNA Amplification Kit is a tool of choice for isothermal amplification of RNA templates and such applications like RT LAMP, 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
- Optimal amplification temperature is 65°C.
- Working temperature range is 55-70°C.
- The Bst enzyme is inactivated in 10 minutes at 80°C.
- RT5 Mix includes thermostable reverse transcriptase (modified MMuLV) working at high temperatures and a thermostable RNase inhibitor protecting template RNA from degradation.
- Optimal 1Step RT amplification reaction time is 30 minutes, if needed, the reaction can be performed 30 to 60 minutes.
- 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 RNA degradation, 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 in a range of 55-70°C for each template/primers system. Complex templates may require higher temperature.
- Suggested reaction time is 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 RT 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:

ALLin™ Isothermal Amplification Mix, 2X	12.5 µl
Optional: Quantitative Fluorescent Dye, 20X	1.25 µl
RT5 Mix (RTase with RNase Inhibitor)	2 µl
10X Primer Mix (variable, depends on application)	2.5 µl
Template RNA (variable, depends on application)	1 µl
PCR Water (supplied)	To 25 µl volume

✓ Mix gently, avoid bubbles.

✓ Place into the thermostat or qPCR instrument to incubate:

Amplification	65°C - 30 min
temperature can be between 55-70°C, time between 20 - 60 minutes	
Optional: Inactivation	80°C - 10 min

Store reactions for short time on ice, for long time at -20°C.

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