ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BC1600400	8 rxns of 50 µl	LyoBead RT-RPA exo 2X RPA Reconstitution Buffer X 20X RPA Reaction Initiator Tube-cap strip
BC1600401	96 rxns of 50 µl	LyoBead RT-RPA exo 2X RPA Reconstitution Buffer X 20X RPA Reaction Initiator Tube-cap strip

COMPONENT	COMPOSITION
LyoBead RT-RPA exo	Optimized and freeze-dried RT-RPA formulation for a reaction of 50 µl
2X RPA Reconstitution Buffer X	Optimized 2X RPA Reconstitution and Reaction Buffer for setting up RPA Master Mix
20X RPA Reaction Initiator	Optimized 20X RPA Reaction Initiator for starting RPA amplification process
Tube-cap strip	Strip of caps for 0.2 ml tubes
STORAGE	Store at room temperature or below (until expiry date)

FEATURES

- 2X Ready-to-use lyophilized master mix with exonuclease III for fluorescence-based real-time detection
- Ultra-rapid monoplex and multiplex amplification of RNA targets
- Robust performance across applications

APPLICATIONS

- Rapid amplification for pathogen detection and environmental testing
- Excellent deployability with minimal lab infrastructure
- Compatible with various readouts, including fluorescence-based real-time measurements
- Suitable for research and assay development

DESCRIPTION

The biotechrabbit™ 2X RT-RPA exo Master Mix delivers fast, reliable RNA amplification with real-time fluorescence detection. It is compatible with standard real-time PCR instruments and any device capable of maintaining a constant temperature while measuring fluorescence signals.
Operating at a constant 37–42 °C, it enables sensitive detection with quantitative results within minutes using an exonuclease III-cleavable probe.

The lyophilized, ready-to-use format requires no cold chain, making it straightforward to deploy in point-of-care and field settings. Pre-formulated for sensitive and specific fluorescence-based detection, it supports real-time monitoring of amplification with clear, reproducible signals and minimal hands-on time, even with complex or unprocessed sample types such as crude extracts. A proprietary reverse transcriptase, engineered for isothermal amplification temperatures, ensures accurate and efficient cDNA synthesis within the reaction.

Robust across a broad range of applications, from clinical diagnostics to environmental monitoring, the 2X RT-RPA exo Master Mix combines speed, stability, and flexibility in a single, easy-to-use solution.

PROTOCOL

RPA primer design

- Suitable RPA primers should be screened using a series of candidate primers and a synthetic dsDNA template.
- RPA primers are longer than conventional PCR primers. Primers should have a length between 30–35 nt (not longer than 45 nt) with a GC content between 30–60 %.
- Software used for PCR primer design may also be used for RPA primer design, such as Primer3.
- Target-specific PCR primer sequences can be used as RPA primers by extending their length.
- For efficient amplification under isothermal conditions, design amplicons between 110–200 bp.
- Shorter amplicons generally yield faster and more robust amplification under isothermal conditions.
- Amplicon lengths should not exceed 400 bp.
- Due to their longer length, RPA primers are more prone to intra- and intermolecular interactions (e.g. hairpins and primer dimers) than typical PCR primers. Such interactions should be avoided for effective primer design.
- The concentration of each primer can be varied between 0.15–0.6 µM.
- The total primer concentration should be in a range of 0.3–2 µM.

RPA Exo probe design

- Suitable RPA Exo probes should be screened using a series of candidate probes, a suitable primer pair and a synthetic dsDNA template.
- The probe should have a length between 46–52 nt.
- The probe must be designed within the amplicon generated by the selected forward and reverse primers and can be oriented in either direction (see fig. 1).
- The probe consists of the following elements:
    - A minimum of 30 bases at the 5’ end.
    - A dT-fluorophore located 3’ of the above 30+ bases.
    - A suitable dT-quencher for the selected fluorophore, positioned 2–5 bases downstream (3′) of the dT-fluorophore.
    - An abasic nucleotide analogue (e.g. tetrahydrofuran (THF) or dSpacer) located between the dT-fluorophore and the dT-quencher.

- At least 15 bases located downstream (3′) of the dT-quencher.
- A 3′ blocker (e.g. Spacer C3 or phosphate) to prevent polymerase extension.

- Identify two thymine residues within the amplicon separated by 2–5 nucleotides. Replace these with a dT-fluorophore and a dT-quencher, respectively. A minimum of 30 bases should be present upstream (5′) of the first thymine, and 15 bases downstream (3′) of the second thymine.
- Replace one base between these two modified thymine residues with a THF or dSpacer.

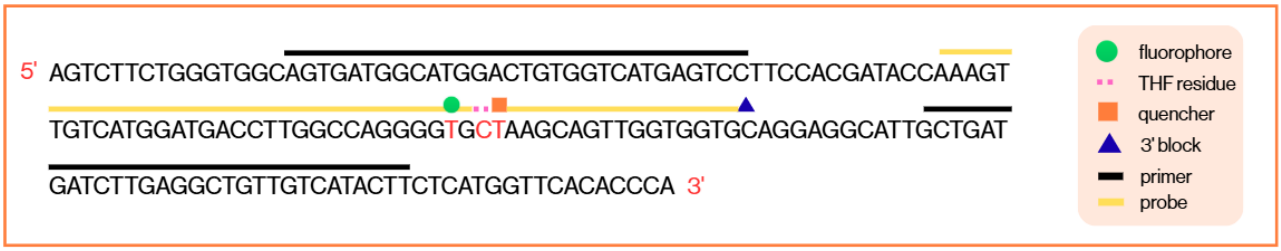

Fig. 1: Example sequence for the 2X RT-RPA exo Master Mix. The positions of forward and reverse primers are indicated by black bars, and the probe position is indicated by a yellow bar. Probe modifications are labelled accordingly. Corresponding bases to be replaced are indicated in red.

The following sequences represent an example of a possible primer and probe design for this assay. The relevant T residues in the probe sequence are replaced by a dT-fluorophore and a dT-quencher, respectively, and one base (C in this case) is replaced by a THF residue. The 3' end is blocked with a C3 Spacer to prevent polymerase extension:

Forward primer (30 nt): AGTGATGGCATGGACTGTGGTCATGAGTCC

Reverse primer (30 nt): AAGTATGACAACAGCCTCAAGATCATCAGC

Exo probe (49 nt): AAAGTTGTCATGGATGACCTTGGCCAGGGG [dT-F] G [THF] [dT-Q] AAGCAGTTGGTGGTG [C3 Spacer]

Prevention of contamination

When assembling the reactions, care should be taken to eliminate the possibility of contamination with undesired RNA, DNA and nucleases.

- Use separate clean areas for preparation of samples and reaction mixtures.
- Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for assay setup.
- Use only water and reagents that are free of RNA, DNA and nucleases.
- With every assay setup, perform a contamination control reaction that does not include a template.

About the 20X RPA Reaction Initiator

The 20X RPA Reaction Initiator triggers the reaction immediately upon contact with the RT-RPA Master Mix. To prevent premature initiation, it is essential to keep the Initiator separate from the RT-RPA Master Mix until just before the reaction begins. For optimal workflow, prepare the final reaction tube containing the RT-RPA Master Mix in advance. Then, pipette 2.5 µl of the 20X RPA Reaction Initiator into the lid of this reaction tube. Avoid mixing the contents yet. Close the tube and briefly spin it down using a mini centrifuge. This step mixes the Initiator with the RT-RPA Master Mix and starts the reaction. Immediately after centrifugation, place the tube into the thermal cycler to begin incubation.

BASIC PROTOCOL

COMPONENT	VOLUME	FINAL CONCENTRATION
LyoBead RT-RPA exo	1 cake	1X
2X RPA Reconstitution Buffer X	25 µl	1X
Primers and Exo probe	Variable	Variable
RNA template	Variable	Variable
Nuclease free water	Add up to 47.5 µl	
Transfer the complete volume of the mix to a reaction tube of your choice, then add (into the tube lid): Alternatively, keep the mix in the original tube and use the provided lid.		
20X RPA Reaction Initiator	2.5 µl	1X
Total volume	50 µl	

- Close the tube and briefly spin it in a centrifuge to initiate the reaction.
- Recommended reaction conditions: 37–42 °C for 10–30 minutes.
- Monitor the fluorescence signals every 30 seconds.
- It is also recommended to try different amplification conditions depending on the target.
- The mix is compatible with a wide range of fluorescence detection instruments.

- NOTE: The tube-cap strip has been included to allow users to securely close the reaction tubes after reconstitution if desired.

RELATED PRODUCTS

2X RPA basic Master Mix, lyophilized (BC1600100)
2X RPA exo Master Mix, lyophilized (BC1600200)
2X RT-RPA basic Master Mix, lyophilized (BC1600300)

LYO-ready Exonuclease III, 100 U/µl (BR1500101)
LYO-ready T6 UvsX DNA Recombinase, 5 mg/ml (BR1500201)
LYO-ready T4 UvsY Protein, 2 mg/ml (BR1500301)
LYO-ready RB69 Gene 32 Protein, 10 mg/ml (BR1500401)
LYO-ready T4 Gene 32 Protein, 10 mg/ml (BR1500501)

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

The performance of the reconstituted LyoBead is confirmed by an RPA reaction.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)

Sicherheitshinweise finden Sie in den Sicherheitsdatenblättern (SDB) unter

www.biotechrabbit.com/support/documentation.html

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.
- In case any customization is required, please contact biotechrabbit via oem@biotechrabbit.com.

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Certified Quality-System mdc ISO 13485 ISO 9001

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