ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BC1600100	8 rxns of 50 µl	LyoBead RPA basic 2X RPA Reconstitution Buffer X 20X RPA Reaction Initiator Tube-cap strip
BC1600101	96 rxns of 50 µl	LyoBead RPA basic 2X RPA Reconstitution Buffer X 20X RPA Reaction Initiator Tube-cap strip

COMPONENT	COMPOSITION
LyoBead RPA basic	Optimized and freeze-dried 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
- Ultra-rapid monoplex and multiplex amplification of DNA targets
- Robust performance across applications

APPLICATIONS

- Rapid amplification for pathogen detection and environmental testing
- Excellent deployability with minimal lab infrastructure
- Compatible with various readouts such as lateral flow and electrophoresis
- Suitable for both research and assay development

DESCRIPTION

The biotechrabbit™ 2X RPA basic Master Mix delivers fast, reliable DNA amplification without the need for thermal cycling. Operating at a constant 37–42 °C, it achieves sensitive detection in as little as 10–20 minutes, accelerating workflows where time matters most.

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 endpoint detection, it delivers consistent, reproducible results with minimal hands-on time, even with complex or unprocessed sample types such as crude extracts.

Robust across a broad range of applications, from clinical diagnostics to environmental monitoring, the 2X RPA basic 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 % (see fig. 1).
- 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 80–200 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.

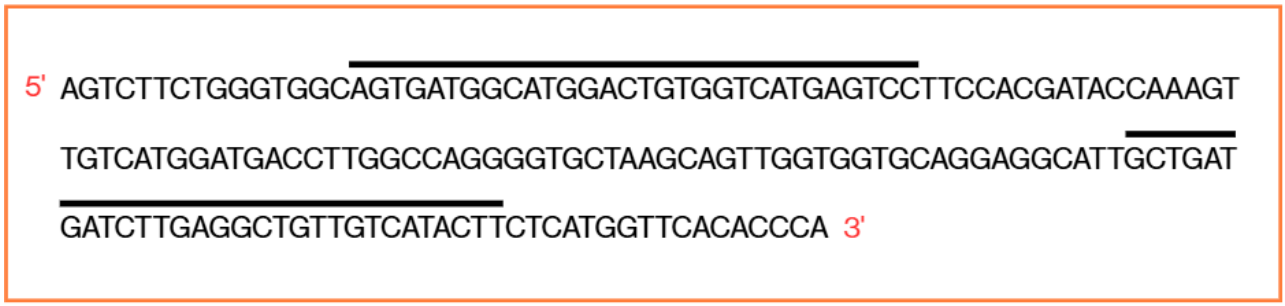

Fig. 1: Example sequence for the 2X RPA basic Master Mix. The positions of forward and reverse primers within the template sequence are indicated by black bars above the sequence.

The following sequences represent an example of a possible primer design for this assay. The forward primer (30 nt) has the sequence AGTGATGGCATGGACTGTGGTCATGAGTCC and the reverse primer (30 nt) has the sequence AAGTATGACAACAGCCTCAAGATCATCAGC.

RPA primer and probe design for lateral flow assay (LFA) - With or without Nfo Probe

Suitable RPA primers without modification (see above) should be screened using a series of candidate primers and a synthetic dsDNA template.

Standard LFA Setup (without Nfo Probe)

- One primer must be labeled with carboxyfluorescein (e.g. FAM) at the 5' end, while the other must be labeled with biotin if an anti-biotin agent (e.g. streptavidin)-coated lateral flow strip with anti-FAM antibody is used.

Optional: Enhanced Specificity with Nfo Probe

If the target specificity of the chosen primer pair is still not sufficient, a target-specific probe should be designed that can be recognized by endonuclease IV (Nfo).

- One primer remains without modification, while the other is designed with a modification at the 5' end (e.g. biotin) to bind to the surface of the lateral flow strip.
- The Nfo probe should be screened using a series of candidate probes 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 must have the same direction as the primer without modification.
- The probe consists of the following elements:
    - The 5' end is labeled with carboxyfluorescein (e.g. FAM).
    - An abasic nucleotide analogue (e.g. tetrahydrofuran (THF) or dSpacer) replaces one of any bases.
    - A minimum of 30 bases located upstream (5') of THF.
    - A minimum of 15 bases located downstream (3') of the THF.
    - A 3' blocker (e.g. Spacer C3 or phosphate) to prevent polymerase extension.

Once the Nfo probe binds to the amplification product that is generated by the primers, endonuclease IV cleaves the THF residue. The truncated sequence labeled with FAM at the 5' end acts as an amplification primer paired with the opposite primer labeled with biotin.

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 RPA Master Mix. To prevent premature initiation, it is essential to keep the Initiator separate from the RPA Master Mix until just before the reaction begins. For optimal workflow, prepare the final reaction tube containing the 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 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 RPA basic	1 cake	1X
2X RPA Reconstitution Buffer X	25 µl	1X
Primers (and Nfo Probe)	Variable	Variable
DNA 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.
- It is also recommended to try different amplification conditions depending on the target.

- 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 exo Master Mix, lyophilized (BC1600200)
2X RT-RPA basic Master Mix, lyophilized (BC1600300)
2X RT-RPA exo Master Mix, lyophilized (BC1600400)

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.

Contact biotechrabbit

biotechrabbit GmbH
Volmerstr. 9
12489 Berlin
Germany

info@biotechrabbit.com
support@biotechrabbit.com
www.biotechrabbit.com

Phone: +49 30 555 78 21 10
Fax: +49 30 555 78 21 99

Certified Quality-System mdc ISO 13485 ISO 9001

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