Lyo-ready RPA Kit

Catalog Numbers A72127, A72128

Pub. No. MAN1000697 Rev. B

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Invitrogen™ Lyo-ready RPA Kit is designed for fast, sensitive, and specific DNA and/or RNA amplification through an isothermal recombinase polymerase amplification (RPA) reaction.

The following reagents are included with the kit as glycerol-free components:

- Lyo-ready T4 UvsX Protein—a homolog of the RecA family recombinases, produced from *E. coli* cells carrying a cloned *uvsX* gene derived from bacteriophage T4. Also available as a standalone product, Cat. No. A72124.
- Lyo-ready T4 UvsY Protein—a recombination mediator protein that stimulates homologous recombination performed by the UvsX recombinase. Also available as a standalone product, Cat. No. A72125.
- Lyo-ready Bst DNA Polymerase—a proprietary mutant of Bst DNA Polymerase, Large Fragment, which exhibits fast reaction speed, increased sensitivity, and tolerance to inhibitors. Also available as a standalone product, Cat. No. A56655.
- Lyo-ready T4 Gene 32 Protein—a single-stranded DNA binding protein, produced from *E. coli* cells carrying a cloned bacteriophage T4 *gp32* gene. Also available as a standalone product, Cat. No. A72123.
- Optimized reaction buffer, magnesium chloride, and dNTPs to provide all the necessary components for DNA amplification. 2X RPA Reaction Buffer is available as a standalone product, Cat. No. A72126 in the event additional reaction buffer is required.

For custom product commercial supply visit **thermofisher.com/mdx**.

Contents and storage

Store all components at –25°C to –15°C.

Component	Cat. No. A72127 (100 reactions)	Cat. No. A72128 (500 reactions)
Lyo-ready T4 UvsX Protein	50 µL	5 × 50 µL
Lyo-ready T4 UvsY Protein	50 µL	5 × 50 µL
Lyo-ready Bst DNA Polymerase	50 µL	5 × 50 µL
Lyo-ready T4 Gene 32 Protein	50 µL	5 × 50 µL
2X RPA Reaction Buffer	1.0 mL	5 × 1.0 mL
$MgCl_2$ (280 mM)	100 µL	5 × 100 µL
dNTP Mix (10 mM each)	50 µL	5 × 50 µL

Required materials

- Template DNA or RNA
- RPA forward and reverse primers
- Nuclease-free water
- 0.2 mL nuclease-free PCR tubes
- PCR thermal cycler or heat block

Optional materials

- SuperScript™ IV Reverse Transcriptase (Cat. No. 18090010)
- RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019)
- RNase H, 5 U/µL (Cat. No. EN0201)
- DNA Loading Dye & SDS Solution (6X) (Cat. No. R1151)
- E-Gel™ Power Snap Plus Electrophoresis System (Cat. No. G9301)
- E-Gel™ EX Agarose Gels, 2% (Cat. No. G401002)
- E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. 10488091)

Important guidelines

- To minimize the risk of environment-borne contamination, clean the working area with a 70% ethanol solution before and after setting up the reactions.
- Use sterile filtered pipette tips to prevent aerosol contamination (see “Contamination prevention” on page 2 for more details).
- Mix all components well and centrifuge the contents down before use to ensure homogeneity.
- Negative control reactions are strongly recommended (e.g., no template control using target-specific primer sets) to demonstrate lack of background amplification.
- Multiple technical replicates of both positive and negative control samples are recommended.
- Lyo-ready RPA Kit components should be thawed and kept on ice during reaction setup.
- RPA and RT-RPA (further referred to as (RT-)RPA) reaction setup should be carried out on ice.
- During (RT-)RPA reaction setup pipette RPA reagents carefully and gently.
- If carrying out end-point detection of (RT-)RPA amplification products, it is recommended to analyze amplicons in a separate workspace than that of the reaction setup.
- Primer design is crucial for successful amplification of the target. Do not use primers shorter than 30 nt (see “General guidelines for (RT-)RPA primer design” on page 2 for more details).
- It is recommended to use nuclease-free, non-stick, low-binding surface tubes for maximal performance. Learn more about preventing RNA degradation at **thermofisher.com/rnase**.
- It is recommended to use SuperScript™ IV Reverse Transcriptase, RNase H, and RNaseOUT™ Recombinant Ribonuclease Inhibitor, or their lyo-ready formats, in reactions containing RNA targets.

Optimization strategies

Template recommendations

- Human genomic DNA range for detection is from 1 ng to 500 ng per 20 µL reaction, although 0.1 ng sensitivity can be achieved.
- Bacteria genomic DNA range for detection is from 0.01 ng to 10 ng per 20 µL reaction, although 0.1 pg sensitivity can be achieved.
- Viral DNA/RNA range for detection is from 100 copies per 20 µL reaction, although 1-5 copy sensitivity can be achieved.

Amplification time

- Optimal amplification time for a (RT-)RPA reaction is 20 minutes.
- For samples with high template input, amplification time can be shortened to 10–15 minutes.
- For samples with low template input and multiplexing the amplification time can be increased to 25 minutes to increase yield and sensitivity.

Amplification temperature

- (RT-)RPA reaction amplification temperature can vary from 34°C to 45°C. The optimal (RT-)RPA reaction temperature is 42°C.
- For samples with high template input, the reaction temperature can be decreased down to 34°C.
- For samples with low template input and multiplexing the reaction temperature can be increased up to 45°C to improve yield and sensitivity.

Product yield

To achieve higher yields, the concentrations of Lyo-ready T4 UvsX Protein, Lyo-ready T4 UvsY Protein, and Lyo-ready T4 Gene 32 Protein can be increased. The optimal final concentration ranges in the reaction mixture for each protein are as follows:

- Lyo-ready T4 UvsX Protein: up to 0.12 mg/mL
- Lyo-ready T4 UvsY Protein: up to 0.045 mg/mL
- Lyo-ready T4 Gene 32 Protein: up to 0.45 mg/mL

RT-RPA reaction

- To achieve highest RT-RPA yield and sensitivity, we recommend using SuperScript™ IV Reverse Transcriptase (Cat. No. 18090010) together with RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019) and RNase H (Cat. No. EN0201).
- Other reverse transcriptases such as Maxima Reverse Transcriptase (Cat. No. EP0741) can be used if maximum sensitivity is not required.

DNA polymerase recommendations

- Bst DNA polymerase concentration can be lowered in the range from 0.015 U/µL to 0.15 U/µL to achieve higher specificity and sensitivity.
- To simplify the addition of a lower concentration of Bst DNA polymerase to RPA reactions we recommend preparing a master mix for multiple reactions. Alternatively, Bst DNA polymerase can be diluted with 1X RPA Reaction Buffer.

Primer concentration

- For single-plex (RT-)RPA reaction 0.3 µM concentration of each primer is recommended.
- For multiplex (RT-)RPA, reducing primer concentration to 0.1 µM each is recommended. If required, primer concentration may be optimized in the 0.1–0.3 µM range.

Shaking

- Shaking at 300 rpm during RPA incubation (i.e., using a thermoshaker) may increase sensitivity.

General guidelines for (RT-)RPA primer design

The following characteristics are of critical importance when designing (RT-)RPA primers.

- Primers for the (RT-)RPA must be ≥30 nt length. Optimal range: 30–35 nt.
- Primers should have 30–70% GC content, avoid single or dinucleotide base repeats, and avoid secondary structure regions.
- Amplicon should have 35–60% GC content.
- Amplicon length should be 150–450 bp.
- Avoid direct/inverted repeats, and palindromes in the target.
- Avoid primer pairs with complementarity at 3' ends.
- If possible, the primer should terminate with a G or C at the 3’ end.

Note: Go to **thermofisher.com/primers** to learn more about ordering primers.

Contamination prevention

It is important to prevent environment-borne and carry-over contamination that can result in non-specific amplification. Observe the following guidelines when performing procedures involving RNA and/or DNA detection.

- Use aerosol-resistant filtered pipette tips only.
- Change pipette tip after each aspiration/dispensation.
- Change gloves frequently during the experiment. Changing gloves after cleaning the laminar flow hood, and prior to adding the NTC is recommended.
- Clean the working area with a 70% ethanol solution before and after setting up the reactions.
- Use separate dedicated areas, equipment, and supplies for:
  - DNA and RNA sample preparation.
  - Reaction setup and amplification.
  - When carrying out end-point detection, make sure the NTC reactions are handled before the positive samples and are closed when positive samples are open.
- If carry-over contamination is suspected, discard used reagents and replace with fresh components.

Perform (RT-)RPA reaction

1 Thaw reagents

Thaw reagents on ice. Carefully mix and centrifuge all reaction components to ensure homogeneity before use.

2 Prepare (RT-)RPA reaction mix for end-point detection

The following procedure provides volumes for a single 20 µL reaction. Multiply the volumes of components common to all reactions by the required number of reactions plus an additional 10% to account for variations in pipetting.

1. Prepare the reaction by adding the components listed in the following table (except $MgCl_2$ and template). Add Nuclease-free water, 2X RPA Reaction Buffer, dNTP Mix, and primers first, mix everything by vortexing, spin down, and add the remaining reagents. While adding proteins, pipette them carefully and gently.

Component	RPA		RT-RPA	
	Volume	Final concentration	Volume	Final concentration
2X RPA Reaction Buffer	10 µL	1X	10 µL	1X
dNTP Mix (10 mM each)	0.4 µL	0.2 mM (each)	0.4 µL	0.2 mM (each)
Forward Primer [1]	Varies based on primer dilution	0.3 µM	Varies based on primer dilution	0.3 µM
Reverse Primer [1]		0.3 µM		0.3 µM
Lyo-ready T4 UvsX Protein (1.2 mg/mL)	0.5 µL	0.03 mg/mL	0.5 µL	0.03 mg/mL
Lyo-ready T4 UvsY Protein (1.2 mg/mL)	0.5 µL	0.03 mg/mL	0.5 µL	0.03 mg/mL
Lyo-ready T4 Gene 32 Protein (16 mg/mL)	0.5 µL	0.4 mg/mL	0.5 µL	0.4 mg/mL
Lyo-ready Bst DNA Polymerase (6 U/µL) [2]	0.5 µL	0.15 U/µL	0.5 µL	0.15 U/µL
SuperScript™ IV Reverse Transcriptase (200 U/µL) [3]	—	—	0.2 µL	2 U/µL
RNaseOUT™ Recombinant Ribonuclease Inhibitor [3]	—	—	0.8 µL	1.6 U/µL
RNase H [3]	—	—	0.4 µL	0.1 U/µL
Template DNA or RNA	Varies	Varies	Varies	Varies
$MgCl_2$ (280 mM) [4]	1 µL	14 mM	1 µL	14 mM
Nuclease-free water	Fill to 20 µL	—	Fill to 20 µL	—

[1] 0.3 µM is recommended primer concentration for single-plex (RT-)RPA reactions. For multiplex (RT-)RPA, reduce primer concentration to 0.1 µM each. If required, primer concentration may be optimized in the 0.1–0.3 µM range.

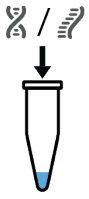
[2] Bst Polymerase concentration in the reaction can range between 0.015–0.15 U/µL. In some cases, lower polymerase concentration can help to achieve higher specificity and sensitivity of target amplicon.

[3] For RNA templates only

[4] $MgCl_2$ has to be added last and separately to each sample ($MgCl_2$ initiates the reaction).

2. Gently vortex the prepared reaction mix and spin it down briefly to collect the droplets off the tube walls.

3 Add template

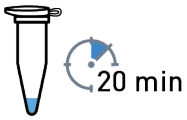


1. Dispense prepared reaction mix (without template and $MgCl_2$)
2. Always start with preparing NTC reactions. Add nuclease-free water to the NTC reaction(s). Close the tubes with NTC before adding the template.
3. Add an equivalent volume of prepared template for the positive reactions, close the tubes, and spin them to collect reaction material.

4 Add $MgCl_2$

1. Add 1 µL of 280 mM $MgCl_2$ solution to the (RT-)RPA reactions and close the tubes.
2. Mix carefully by vortexing briefly, or tapping the tube with your finger.
3. Spin the tubes to collect reaction material at the bottom of the tubes.

5 Run the (RT-)RPA reaction



Run the reaction on thermal cycler or heat block(s) according to following conditions.

Step	Temperature	Time
Amplification	42°C	20 minutes
Hold	4°C	Hold

Note: If a thermal cycler is used for amplification, open lid is recommended.

Postamplification procedures

Analyze RPA product by gel electrophoresis

It is recommended to open (RT-)RPA tubes in a workspace separate from the reaction set-up area.

After amplification, run samples on agarose gel to visualize the presence of amplicons. The following procedure is for using E-Gel™ EX Agarose Gels, 2% (Cat. No. G401002) with the E-Gel™ Power Snap Plus Electrophoresis System (Cat. No. G9301). Other types of agarose gels can be used according to the specific needs of the user.

1 Prepare samples

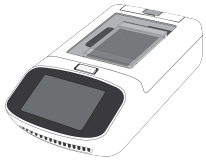
Note: Prepare negative control samples first to avoid the contamination of the negative control with amplicons from the positive reactions.

1. Add DNA Loading Dye & SDS Solution (6X) (Cat. No. R1151) to (RT-)RPA product, mix, and briefly centrifuge the contents.
2. Incubate samples at 65°C for 10 minutes.
3. Dilute the sample 30-fold by combining 2 µL of the sample with 58 µL of nuclease-free water. Mix the samples by flicking the tube or gently vortexing and spin the samples down.

2 Prepare DNA ladder

Dilute the E-Gel™ 1 Kb Plus Express DNA Ladder 10–60 fold. Gently mix and briefly spin down.

3 Run gel



1. Transfer 20 µL of ladder and samples (starting with the negative controls) into the wells of the E-Gel™ agarose gel.
2. Load 20 µL of deionized water in all empty wells.
3. Run electrophoresis according to the selected program and analyze the obtained results.

Other detection methods

(RT-)RPA is compatible with real-time, and CRISPR-Cas systems.

Troubleshooting

Observation	Possible cause	Recommended action
Low product amount or no product	Problem with reaction mix.	Repeat the experiment to make sure there were no pipetting errors.
		Make sure all the reaction components are thawed and mixed well prior to use.
		Check primer length, complementarity, and specificity to the target nucleic acid. Redesign primers if necessary. Adhere to (RT-)RPA primer design rules.
		Increase the amount of template. Note that template amounts outside the recommended range can inhibit the reaction.
		Increase the amount of T4 UvsX, T4 UvsY, and T4 Gene 32 protein added per reaction (keep within the recommended concentration ranges in “Optimization strategies” on page 2).
		Reduce the amount of Bst polymerase added per reaction, within the concentration range suggested in the protocol.

Observation	Possible cause	Recommended action
Low product amount or no product *(continued)*	Sub-optimal amplification parameters.	Prolong reaction time.
		Increase incubation temperature to 45°C.
Non-specific products	Sub-optimal reaction mix parameters.	Reduce the amount of Bst polymerase added per reaction, within the concentration range suggested in the protocol.
		Make sure that $MgCl_2$ is added last.
	Sub-optimal amplification parameters.	Lower amplification temperature.
False-positive result	Environment-borne and/or carry-over contamination.	Follow recommended cleaning instructions prior to and after each experiment.
		Regularly clean used equipment and the working area.
		If contamination of equipment is suspected, clean the heating block of the thermal cycler or thermostat.
		Always wear gloves while performing the experiment.
		Use sterile filtered pipette tips only.
		In case of end-point detection, analyze amplicons in a workspace separate from the reaction setup area.
		Prepare negative controls in an area separate from where test samples are added to the reaction.
		Close NTC tubes prior to adding target.
		Replace potentially contaminated reagents with new ones. Use a new tube of nuclease-free water for each experiment.
		Aways follow tips from “Important guidelines” on page 1 and “Contamination prevention” on page 2 to minimize contamination risk.

Documentation and support

Customer and technical support

Visit our product page at **thermofisher.com** for additional information and protocols.

For support, visit **thermofisher.com/support**.

For additional resources on isothermal nucleic acid amplification, visit **thermofisher.com/isothermal**.

Limited product warranty

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Revision history: Pub. No. MAN1000697 B

Revision	Date	Description
B	6 March 2025	Removal of statement in text.
A	9 October 2024	New document for the Lyo-ready RPA Kit.