EZassay

RT-RPA Basic kit
(UDG Enzyme Based Contamination Control)

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Catalog Code: BA-RT-LYO-FW-48
BA-RT-LYO-FW-96

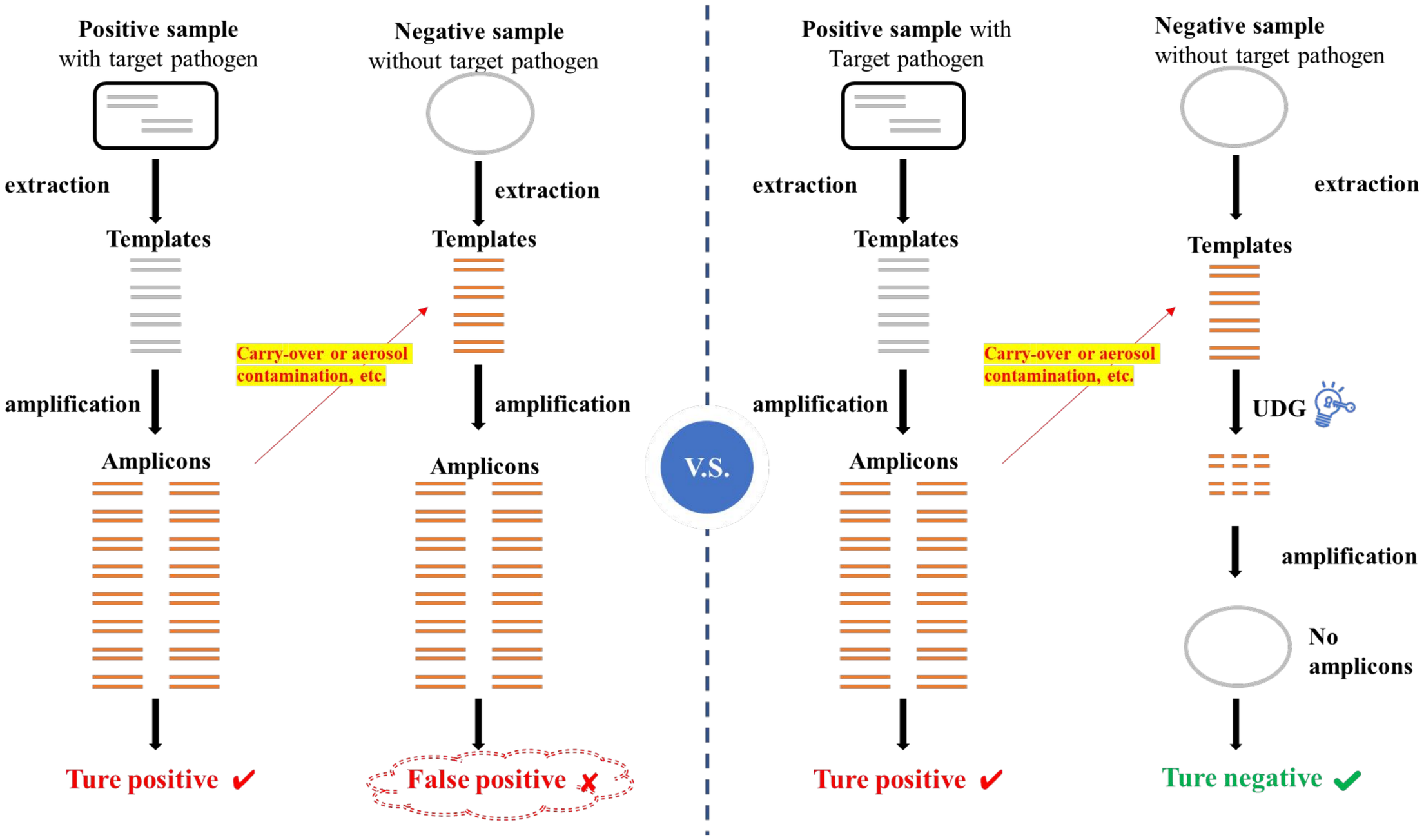
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Brief introduction

This kit provides the essential components required for recombinase polymerase amplification (RPA) of nucleic acids. It offers high sensitivity, strong specificity, and rapid amplification. To reduce contamination risks, this product offers a thermolabile UDG enzyme. It can quickly, efficiently, and specifically degrade amplification products, thus preventing cross-contamination or aerosol contamination caused by them.
The reaction is performed at a constant temperature of 37–42 °C, with amplification typically completed within 30 minutes.

The kit features a simple workflow and minimal equipment requirements. Reactions can be carried out using standard constant-temperature devices such as a heat block or water bath, without the need for a thermal cycler, making it suitable for rapid and point-of-care nucleic acid testing.



Materials supplied

Item	BA-RT-LYO-FW-48	BA-RT-LYO-FW-96
RPA Reaction Tubes*	48T	96T
Rehydration Buffer (2X)	500μL	500μL*2
Positive control (10X)	10μL	10μL *2
Starter (10X)	100μL	100μL*2

UDG (10X) (lyophilized)**	1 vial	2 vial
UDG Diluent Buffer	1000μL	1000μL*2

*Contains RPA lyophilized pellets. Briefly centrifuge before use to ensure the lyophilized pellet is collected at the bottom of the tube.
**Reconstitute the UDG lyophilized pellet by adding the volume of UDG Diluent Buffer indicated on the tube label. Mix thoroughly until completely dissolved.

Required materials but not supplied

1. RPA primers (online software: https://www.ezassay.com/primer)
2. Incubator such as PCR thermal cycler, heat block or water bath
3. Nuclease-free water
4. RNA template

Storage

-20°C

Assay procedure

1. Set incubator working temperature at 39 °C. (Optimization range 37~42°C)
(Turn off the lid heating function or set to 42 °C if PCR thermal cycler is used.)
2. Sample Processing.
Extract or release nucleic acids from the sample to serve as the template for the amplification reaction.
To reduce the risk of carryover contamination, treat the template with UDG. Mix 9 μL of template with 1 μL of 10× UDG, then incubate at room temperature (25–37°C) for 2–5 minutes.
3. For each reaction tube, add reagents as described in the table below. It is recommended to set up the reactions on ice. To minimize pipetting variation, prepare a master mix according to the total number of reactions.

Component	Sample group	Positive control group	Negative control group
Rehydration Buffer(2x)	10μL	10μL	10μL
Forward Primer (20μM) Reverse Primer (20μM)	0.5μL 0.5μL	- -	0.5μL 0.5μL
RNA template	x μL	-	-
Positive control (10X)*	-	2μL	-
Nuclease-free H2O	Up to 18μl		

* The Positive Control contains primers and the RNA template.

4. Dilute the UDG (10 X) with UDG Diluent Buffer. Add 1 μL of the diluted UDG (1X) to the inside of the tube cap.

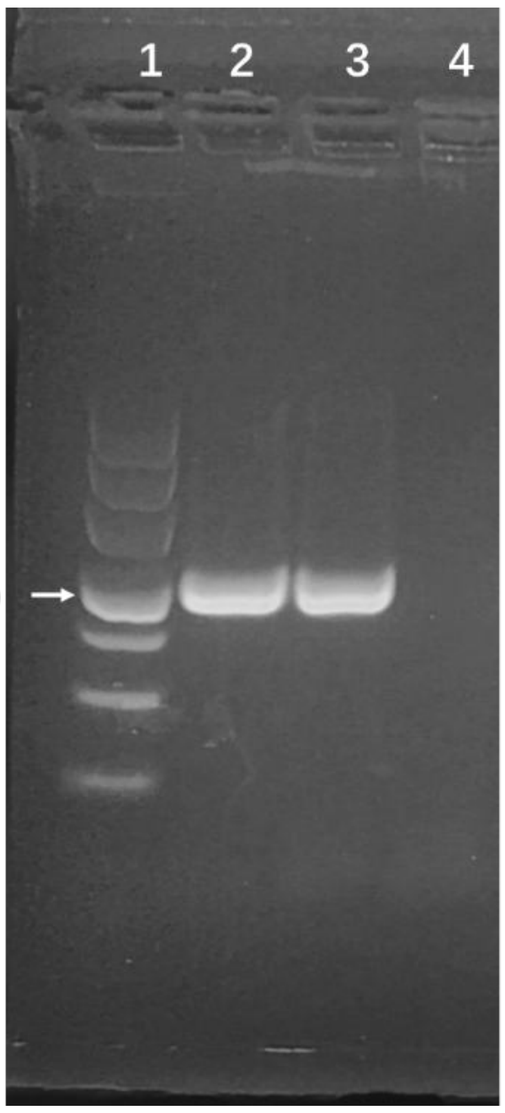
5. Add 2 μL of Starter to each RPA Reaction Tube. It is recommended to dispense the Starter onto the inner side of the tube cap or the tube wall.

6. Invert and flick the reaction tube, then briefly centrifuge. Repeat 3 times to ensure thorough mixing. The lyophilized pellet in the RPA Reaction Tube should be completely dissolved and evenly mixed. Avoid vigorous vortexing.

7. Incubate at 39°C for 20~40 minutes to generate sufficient amplicons.

8. The reaction can be terminated by heating at 65°C for 10minutes (please note that high-temperature denaturation does not effectively remove proteins, which may interfere with downstream product analysis). Then take 1–10 μL of the amplification products for agarose gel electrophoresis detection (a gel concentration of 2%–3% is recommended).

It is recommended to purify the amplification products using a DNA purification kit before agarose gel electrophoresis analysis. (Note: some commercially available amplification product purification kits are not compatible with this system and may cause false-negative results)



Example of agarose gel. The size of amplicon of positive control is 200bp.

Notes

- It is recommended to implement physical separation between pre-amplification and post-amplification areas. Independent workspaces, equipment, and consumables should be used for sample preparation, reaction setup, and amplification procedures to minimize the risk of amplicon contamination.
For endpoint detection, ensure that the negative control (NTC) is handled prior to positive samples, and keep the negative control tubes tightly closed when opening positive sample tubes.
If amplicon contamination is suspected, discard the used reagents and replace them with fresh reagent components.
- For samples with low template concentrations, the reaction tube may be gently flicked several times and briefly centrifuged at the 4-minute time point to improve mixing (avoid vigorous shaking), then returned to the original position to continue the reaction.
- Please note that there may be differences between the set temperature and the actual reaction temperature. For first-time use, it is recommended to perform a temperature gradient test, for example by setting up three reactions at 37 °C, 39 °C, and 42 °C to determine the optimal reaction temperature.
- If PCR thermal cycler is used, please make sure to turn off the heat lid function or set it to 39 °C. For some types, the lid temperature goes up to 105°C immediately after power on. The lid is already hot enough to inactivate enzymes. Please run a few cycles to allow the temperature going down.
- 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 beachieved.

¢ Viral DNA/RNA range for detection is from 100 copies per 20 μL reaction, although 1-5 copy sensitivity can be achieved.

- Primer Concentration

¢ For singleplex (RT-)RPA reactions, a final concentration of 0.3–0.6 μM for each primer is recommended.

¢ For multiplex (RT-)RPA reactions, it is recommended to reduce the concentration of each primer to 0.1 μM. If necessary, primer concentrations may be further optimized within the range of 0.1–0.3 μM.

- All components should be completely dissolved and mixed.
- The template and Starter should be added separately to different areas of the reaction tube and then centrifuged to the bottom of the tube.
- For samples with low template concentrations, flick them several times in the 4th minute of the reaction, centrifuge and mix well (avoid vigorous shaking), and then return to the original well position to continue the reaction.

Design principles of basic primers for isothermal amplification

1、 Primer length: 30-35nt.

2、 The first 3-5 nucleotides of the 5' end of the primer avoid consecutive G bases, but if it is a C base or T base, it is good for amplification, and the primer will help the recombinase and the primer to form a filament structure.

3、 The last three bases at the 3' end of the primer, with G and C, are conducive to amplification, probably because the anchoring of the polymerase makes the binding of the enzyme and primer more stable.

4、 GC content of primer should be greater than 30% and less than 70%.

5、 The length of the amplicon should not exceed 500 bp, preferably around 150-250 bp.

6、 There are also general primer design principles:

1) Avoid more than 4 single bases in a row;

2) It should not contain its own complementary sequence, otherwise it will form a hairpin-like secondary structure;

3) There should be no more than 4 complementary or homologous bases between the two primers, otherwise primer dimers will be formed, especially the complementary overlap of the 3' end should be avoided;

4) 3' ends: If possible, the 3' end bases of each primer should be G or C, but there should be no more than 3 consecutive Gs or Cs at its 3' end, otherwise the primer will be mis-raised in the GC-enriched sequence region.

7、 An example of a primer screening process：

1) The first round of primer screening was carried out, and the forward F3 primer was selected to screen all the reverse primers, and the optimal result was F3+R4, and R4 was determined to be the optimal reverse primer;

	R1	R2	R3	R4	R5
F1					
F2					
F3	--	+	+	+++	++
F4					
F5					

2) Perform the second round of primer screening, and use R4 as the optimal reverse primer to screen all the forward primers to obtain the most. The optimal result is: F4+R4.

	R1	R2	R3	R4	R5
F1				--	
F2				++	
F3	--	+	+	+++	++
F4				++++	
F5				++	

8. When screening primers, they should be screened under relatively 'difficult reaction conditions', such as low template concentration and short reaction time.
9、The concentration of primers can be optimized between 150 nM and 600 nM.
10、Primer screening can be performed directly using an agarose gel to see if the fragment of interest can be amplified.

Choose the right product

Product name	Classify	Template	Catalog number	Brief introduction
RPA/RAA Isothermal amplification	Basic kit	DNA	BA-LYO-96	Similar to PCR, it is mainly used to complete DNA amplification. You can observe the results with DNA gel. You can also combine it with CRISPR technology.
		RNA	BA-RT-LYO-96	
	Exo kit	DNA	EX-LYO-96	Based on the basic kit, a fluorescent probe (Exo probe) is introduced. It is similar to fluorescent probe PCR. Fluorescent signals can be read with a fluorometer.
		RNA	EX-RT-LYO-96	
	Nfo kit	DNA	NF-LYO-96	Based on the basic type, lateral flow probe (Nfo probe) is introduced. Test results can be observed with lateral flow paper strips.
		RNA	NF-RT-LYO-96	

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