EZassay

RPA Nfo Kit

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Catalog Code: NF-LYO-48
NF-LYO-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.
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.
The amplification results can be visually detected using a lateral flow strip, enabling rapid and instrument-free result interpretation.

Materials supplied

Item	NF-LYO-48	NF-LYO-96
RPA Reaction Tubes	48T	96T
Rehydration Buffer (2X)	500μL	500μL*2
Positive Control* (10X)	10μL	10μL*2
Starter (10X)	100μL	100μL*2

Required materials but not supplied

1. RPA primers and probe (online software: https://www.ezassay.com/primer)
2. Incubator such as PCR thermocycler, heat block or water bath
3. Nuclease-free water
4. DNA template (RNA needs to be reverse-transcribed into DNA first)
5. Nucleic acid amplicons detection strip (Recommend EZassay product. Cat.#. PS-FMBO-96)

Storage

-20°C

Assay procedure

1. Set the qPCR working temperature at 39 °C. (Optimization range 37~42°C) (Turn off the lid heating function or set to 42 °C.)
2. 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)	0.42μL 0.42μL	- -	0.42μL 0.42μL
Nfo probe (4μM)	0.6μL	-	0.6μL
DNA template	x μL	-	-
Positive control (10X)*	-	2μL	-
Nuclease-free H2O	Up to 18μL		

* The Positive Control contains primers, the Nfo probe, and the DNA template. The probe is labeled with FAM-Biotin.

3. 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.
4. 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.
5. Incubate at 39°C for 20~40 minutes to generate sufficient amplicons.
6. Take 10 μl of the amplification product, add 80 μl of Diluent Buffer, mix well, and add 70 μl to the test strip. Note. The Diluent Buffer is provided together with the lateral flow strip. The dilution factor can be optimized according to the actual situation, generally between 1:9 and 1:500.
7. Read the results

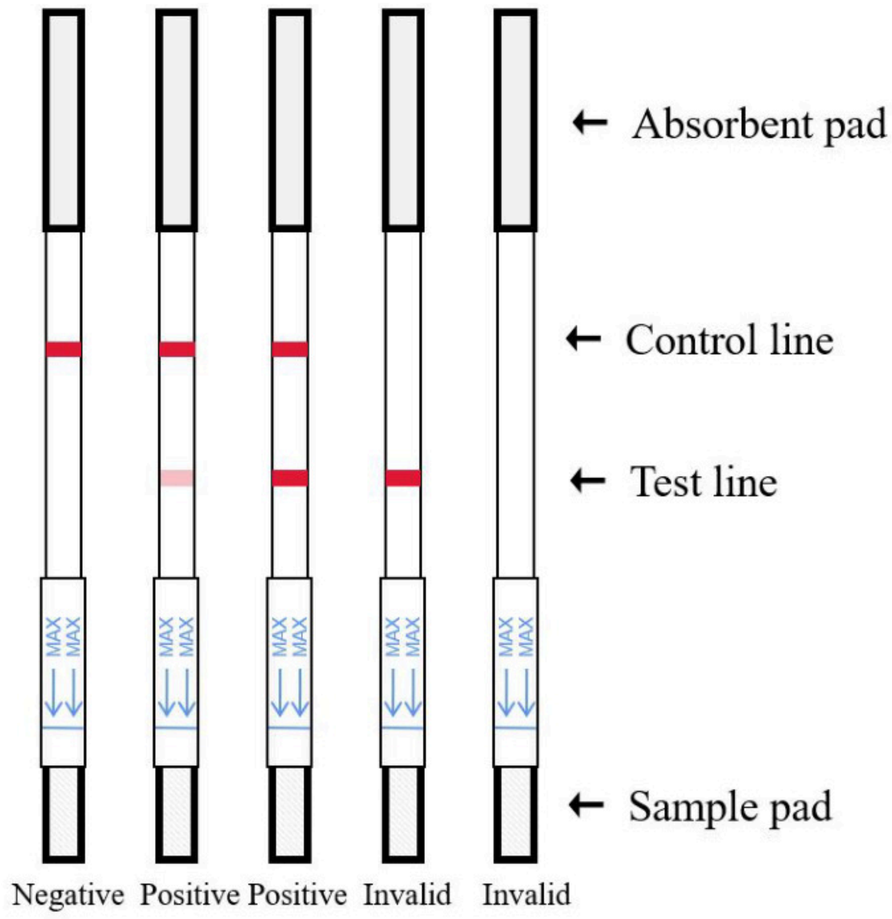


Figure 1. Schematic diagram of the interpretation of the results

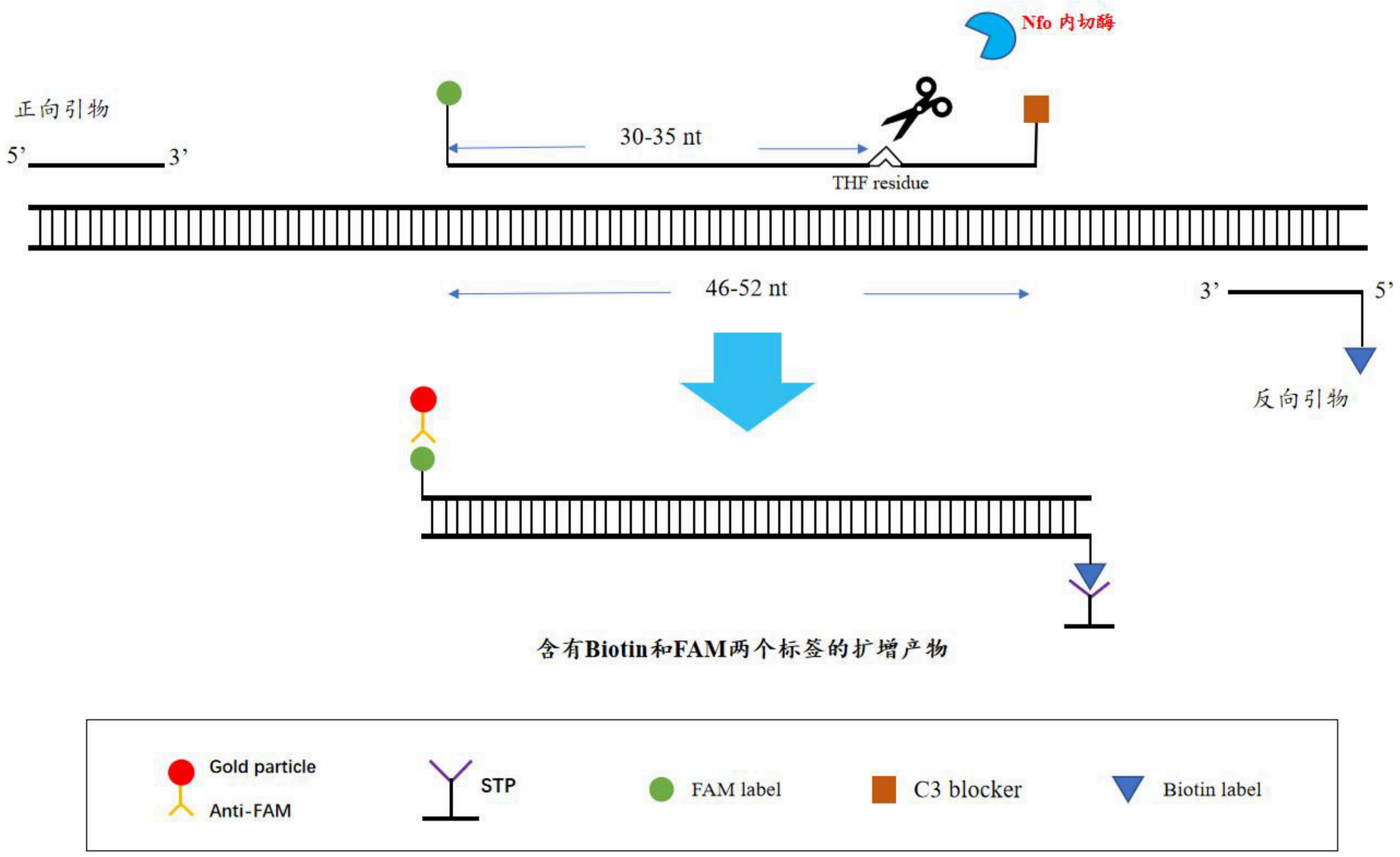
Notes

- 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 41°C to determine the optimal reaction temperature.
- If qPCR instrument is used, please make sure to turn off the heat lid function or set it to 45 °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.

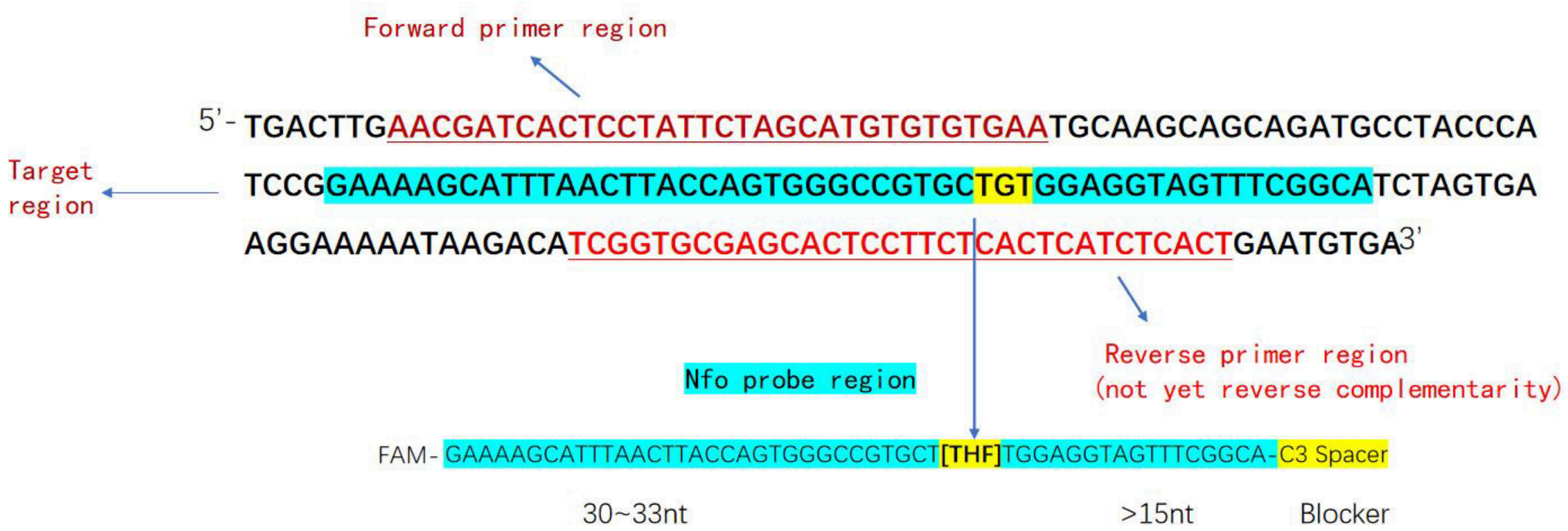
The DNA template and starter should be added separately to different areas of the reaction tube and then centrifuged to the bottom of the tube.

Schematic diagram of working principle of Nfo kit



Design principles of probe for Nfo kit

1. Nfo probes typically contain nucleotide analogues THF (tetrahydrofuran), fluorophores (FAM/TAMRA, etc.) and 3'-terminal blocks (suitable 3'-modified groups (e.g., C3-spacer, a phosphate, a Biotin-TEG or an amine). Example of Nfo probe:



2. Probe length: normally among 46-52nt;
3. The distance between the 5' end of the probe and THF site is 30-33nt;
4. The distance between the 3' end of the probe is ⩾ 15nt from THF;
5. The distance between the fluorophore and the quencher group should be ⩽5nt;
6. The distance between the fluorophore and THF site, the quencher group and THF site should be between 0~2nt, and the maximum should not exceed 2nt, otherwise the cleavage effect of Exonuclease will be poor;
7. The 5' end of reverse primer needs to be biotin modified.
8. Please note that the 3'-block of the Nfo probe cannot be blocked with biotin!
9. Primers in the same direction can overlap with the probe, and the overlapping part is less than 20nt.
10. Avoid the formation of dimers by probes and primers.
11. The concentration of the probe can be optimized between 50 nM and 150 nM;
12. The primer design of Nfo kit is the same as basic kit. The probe design can be carried out after primer screening. Probe should be between the upstream and downstream primers. Over-lap can be accepted.
13. It is recommended to perform negative control and positive control as needed.
14. Please note that amplicons will generally be cut by Nfo nuclease. There is no clear band when you run DNA gel.

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	