PrimeIAmp™ Fast Probe RT Isothermal Amplification Kit

User's Instruction

Description

PrimeIAmp™ Fast Probe RT Isothermal Amplification Kit, with fast isothermal amplification technology, single molecule fluorescence detection technology and reverse transcription technology, achieves RNA reverse transcription and amplification as well as real-time detection of the specific RNA in a single system. Compared with conventional real-time fluorescent RT-PCR which takes about 2 hours, this kit can determine whether there is a specific RNA in a sample within 20 minutes. The detection process does not need to open the cover, which avoids the generation of aerosol, greatly improving the reliability of the results.

Kit Contents

	96 T
1. Dissolving Reagent	1.2 ml×2
2. Activator	250 μl
3. Positive Control Primers	180 μl
4. Positive Control Probes	15 μl
5. Positive Standards	80 μl
6. PrimeIAmp™ Fast Probe RT MasterMix	24T×4

Features

- Rapid amplification: In most cases, trace nucleic acid samples can be amplified to detectable level within 20 minutes.
- High stability: The product is freeze-dried and can be transported at room temperature. It can be stored at -20°C for more than one year.
- High sensitivity: The detection limit can reach 10-100 copies/reaction.
- Easy operation: the main components of the product are prefabricated into microspheres by advanced freeze-drying process, without the need of professional equipment and training. The whole process is easy to operate.

Primer Design

The principle and algorithm are similar to PCR primer design:

- Please use primers with a length of 30-35 bp. Short primers may affect the amplification rate and detection sensitivity.
- Primer design should avoid the formation of secondary structure, which may affect the amplification.
- Primer Tm value should be 50-75, and GC content should be 30%-70%.
- The 5' end of the primer should avoid G while the 3' end of the primer should contain G and C.
- The length of amplicon is recommended to be 100-200 bp

Protocol (Test Sample)

1. Set up the premix as the following table:

Component	Volume
Dissolving Reagent*	20 μl
Forward Primer (10 μM)	2.1 μl
Reverse Primer (10 μM)	2.1 μl
Probes (10 μM)	0.6 μl
Template RNA + ddH_2O	23.2 μl

*Note: Dissolving Reagent needs to be fully melted and mixed.

2. For each sample, add 48 μl of the premix into each PrimeIAmp™ Fast Probe RT MasterMix. Shake and mix until the mix is resuspended and then centrifuge transiently.
3. For each sample, add 2 μl activator to the reaction tube cover. Carefully close the tube cover, and allow the activator to enter the mix by transient centrifugation. Shake and mix until the mix is resuspended and then centrifuge transiently again.
4. Put the reaction tube into a constant temperature fluorescence detector (40°C), start the detection for 20 minutes, and collect the fluorescence value of fam channel every 30 seconds.
    a. Note: If using ABI series PCR instrument, be sure to select "None" at both passive reference and quencher.

Protocol (Positive Control)

PrimeIAmp™ Fast Probe RT Isothermal Amplification Kit includes positive control primers, probes and positive standards, which can be used to detect the activity of each component

in the kit. The detection method is the same as the above protocol. The positive control probe is labeled with fluorescein (FAM). The optimal excitation wavelength is 492 nm and the maximum emission wavelength is 520 nm.

1. Set up the premix as the following table:

Component	Volume
Dissolving Reagent*	20 µl
Positive Control Primers	4.2 µl
Positive Control Probes	0.6 µl
ddH_2O	21.2 µl
Positive Standards**	2 µl

*Note: Dissolving Reagent needs to be fully melted and mixed.
**Note: 2 µl - 5 µl is recommended.

2. For each sample, add 48 µl of the premix into each PrimeIAmp™ Fast Probe RT MasterMix. Shake and mix until the mix is resuspended and then centrifuge transiently.
3. For each sample, add 2 µl activator to the reaction tube cover. Carefully close the tube cover, and allow the activator to enter the mix by transient centrifugation. Shake and mix until the mix is resuspended and then centrifuge transiently again.
4. Put the reaction tube into a constant temperature fluorescence detector (40°C), start the detection for 20 minutes, and collect the fluorescence value of fam channel every 30 seconds.
    a. Note: If using ABI series PCR instrument, be sure to select "None" at both passive reference and quencher.

Additional Notes

1. Blank control without template should be set in the experiment to confirm whether the nucleic acid to be amplified is contaminated.
2. To avoid cross contamination, reagent preparation area and amplification analysis area should be separated.
3. Under different nucleic acid extraction methods, the content and purity of RNA extracted from samples will be different, which may lead to the differences in amplification efficiency
4. If the copy number of template RNA is low, take out the reaction tube after 5 minutes of reaction, shake and mix well, centrifuge briefly, and then put it back into the constant temperature fluorescence detector.

Storage

Minimum shelf life is 1 years at -20°C. For positive standard, please store at -80°C.