PrimeIAmp™ Red Lyophilized Isothermal Amplification Microbeads User's Instruction

Description

PrimeIAmp™ Red Lyophilized Isothermal Amplification Microbeads contain reaction buffer, red-yellow colorimetric visible indicator, Mg^{2+}, dNTP, Bst DNA/RNA polymerase and so on in lyophilized form. Only primers and templates are needed to be added for the isothermal amplification. After amplification, the positive samples are yellow, while the negative samples are red. The results can be directly observed by naked eyes without any other auxiliary equipment. PrimeIAmp™ Red Lyophilized Isothermal Amplification Microbeads can be stored at room temperature (25°C) for 6 months.

Bst DNA/RNA Polymerase is a mixture of Bst polymerase and extremely thermostable reverse transcriptase (65°C tolerant), which is suitable for isothermal amplification reaction of RNA. It can detect low-sensitivity RNA molecules. This enzyme is recommended in isothermal amplification experiments using RNA as a template. In addition, Bst DNA/RNA Polymerase can also perform isothermal amplification of DNA templates.

Kit Contents

	100 T
PrimeIAmp™ Red Lyophilized Microbeads	100 pcs

Protocol

1. Set up isothermal amplification reaction as the following table:

Component	Volume
PrimeIAmp™ Red Lyophilized Microbeads	1 pc
*10 × Primer Mix	2.5 μl
Template (DNA/RNA)	X μl
ddH_2O	Up to 25 μl

*10 × Primer Mix：16 μM FIP/BIP, 2 μM F3/B3, 4 μM LoopF/B each.

2. After the reaction system is prepared, carry out the reaction at 60 ~ 68°C (65°C is recommended for the first experiment) for 15 ~ 25 min. The results can be observed by naked eyes. The positive samples are yellow, while the negative samples are red.

Storage

Minimum shelf life is 6 months at 25°C and 3 years at 2-8°C. The product can be transported at room temperature. After the product is opened, the unused product should be sealed with sealing film or electrical tape to prevent the performance degradation due to moisture. The product can also be vacuum packed in 0.2 ml EP tube.

Note

- This red yellow discoloration reaction depends on the change of pH in the reaction system. Therefore, Tris salt, NaOH and other components in the template will have impact on the reaction. We recommend to use ddH_2O for elution when using nucleic acid purified samples.
- We recommend use ddH_2O to preserve samples for detection when using crude samples. The best crude sample is swab sample. Swab sample soaked in ddH_2O can be directly used as template for amplification without nucleic acid purification.
- When used in other isothermal amplification reactions (such as CPA, SMAP, etc.), the above strategies can be referred while the reaction time may need to be adjusted.
- After the reaction system is prepared, a drop of mineral oil can be added to cover the upper part of the reaction liquid to reduce aerosol pollution.
- Preparation of complete amplification system using lyophilized microbeads: Pack optimized primers at the bottom of 0.2 ml EP tube and dry at 70 ~ 80 °C for 1 ~ 2h. The dried 0.2 ml EP tube already contains dried amplification primers, and then add a lyophilized microbead to prepare the complete amplification system, which can be stored for a long time, without the need of cold chain transportation.

Only for research and not intended for treatment of humans or animals