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Direct Start Probe qPCR mix(UDG, for-Lyo)

Product Number: PCK009LY

Shipping and Storage

-20℃.

Components

Component	PCK009LY
2×Direct Start Probe qPCR mix(UDG, for-Lyo)	5ml
RNase Free Water	5ml
ROX I	200μl
ROX II	200μl

1. The Real Time PCR instrument calibrated with ROX I includes:
   ABI 5700/7000/7300/7700/7900/7900 HT/7900 HT Fast,ABI StepOne/StepOnePlus and other instruments.
2. The Real Time PCR instrument calibrated with ROX II includes:
   ABI 7500/7500 Fast;ABI ViiA7;ABI Q6,ABI Quant Studio 6/7 Flex;Stratagene MX4000/MX3005P/MX3000P and other instruments.
3. Real Time PCR instruments that do not require ROX calibration include:
   Bio-Rad CFX96/CFX 384/iCycler iQ5/iQ/My iQ5/MiniOpticon/Opticon/Opticon 2/Chromo4； Eppendorf Mastercycler ep realplex /realplex 2s；Cepheid SmartCycler；Illumina Eco qPCR；Roche Applied Science LightCycler 480；Thermo Scientific PikoReal Cycler； Qiagen/Corbett Rotor-Gene Q/Rotor-Gene 3000/ Rotor-Gene 6000 and other instruments.

Description

Direct Start Probe qPCR mix (UDG, for Lyo) is a specialized reagent designed to directly perform fluorescence quantitative PCR detection (TaqMan or Molecular Beacon, etc.) without the nucleic acid extraction and purification steps of samples. This product has strong inhibitor tolerance and does not require DNA extraction and purification. It can be used for direct DNA amplification detection of anticoagulant whole blood, plasma, serum and other samples. The dUTP-UDG contamination prevention system was applied, and dUTP was added during the PCR reaction to form amplification products containing dU bases. This product can be digested by UDG enzymes in the system before the next PCR reaction, effectively preventing false positives caused by PCR product contamination.

This product is a liquid reagent pre mixed with HotStart Taq DNA polymerase, UDG enzyme, optimized reaction buffer, dNTPs, protectants, excipients, and other reagents. The liquid reagent does not contain glycerol or other components that affect freeze-drying and can be used for the preparation of freeze-dried samples to improve the stability and usability of the samples. When conducting experiments, primers, probes, templates, and water can be added to prepare the PCR reaction solution. This product is prepared by a special process using HotStart Taq DNA polymerase and combined with a specially optimized reaction and freeze-drying formula to produce a freeze-drying reagent. It can effectively inhibit non-specific reactions, improve reaction specificity and amplification efficiency, and accurately quantify over a wider range. And equipped with ROX Reference Dyes of different concentrations, used for correcting inter well fluorescence signal errors with different instruments.

Features

1. This product can be used to make freeze-dried powder and freeze-dried balls;
2. The reaction buffer system formulated with HotStart Taq DNA polymerase, manufactured using a special process, can tolerate various inhibitors in the blood and can tolerate 10% whole blood template;
3. Wide amplification range, capable of detecting fresh and frozen blood from various anticoagulants;
4. The dUTP-UDG system effectively prevents false positives caused by PCR product contamination;

5. The necessary reagents for PCR reaction are collected in one tube, and the preparation of the reaction system can be completed in a few minutes.

Quality control

Purity testing: All components have been tested and found to have no residual endonucleases or exonucleases.

Protocol

1. Preparation of freeze-dried microsphere system
   1.1. According to the following commonly used systems (20μl) Preparation of freeze-dried ball system:

Component	Volume	Final Conc.
2×Direct Start Probe qPCR mix(UDG, for-Lyo)	10μl	1×
10×Primer Mix*	2μl	1×
RNase Free Water	Up to 20μl	

*10×Primer Mix includes 2-10μM upstream primers, 2-10μM downstream primers and 5μM probe.

   1.2. Freeze dry the above liquid samples;
   1.3. After freeze-drying, the sample was re dissolved in (20-X)μl RNase Free Water, and then reacted with Xμl's template. The total volume of the reaction was 20μl;
2. Preparation and use of freeze-dried powder samples
   2.1. Prepare freeze-dried samples of 2×Direct Start Probe qPCR mix (UDG, for Lyo) according to requirements;
   2.2. Dissolve the sample prepared by freeze-drying in the corresponding volume of RNase Free Water before use;
3. Referral process

Step	Temperature	Time	Cycles
Pollution digestion	50℃	2min	1
Pre denaturation	95℃	5min	1
Denaturation	95℃	10s	35-45
Annealing/Extension	60℃	30-60s	

Note

1. Before use, make sure to mix thoroughly to avoid excessive bubbles caused by violent shaking;
2. qPCR has extremely high sensitivity, it is recommended to dilute the template for use;
3. The recommended amount of blood template usage is 10% of the total reaction volume, which is 20 μ Add 2ul of whole blood as a template to the reaction system, taking care to avoid absorbing blood clots;
4. It is recommended that the three processes of reagent premixing, template addition, and detection be carried out in different enclosed areas as much as possible, and use gun heads and reaction tubes without nuclease residues;
5. Recommended use the gun head with filter element. Avoid cross contamination and aerosol contamination;
6. This product already contains freeze-dried excipients, which can be directly freeze-dried. If other freeze-dried excipients need to be added, they need to be evaluated based on actual testing data before being added.