NO：CE3002

SYBR Green/SYBR Premix Ex Taq Kit

Introduction

SYBR Premix Ex Taq Kit is used for intercalator based real time PCR using SYBR®Green. Rapid reaction mixtures are based on a 2×concentration premixed with SYBR®Green I at a concentration appropriate for real time monitoring.

A combination of Ex Taq™ HS, a hot start PCR enzyme that uses an anti-Taq antibody, and a buffer optimized for real time PCR allows high amplification efficiency and high detection sensitivity in real time PCR. The 2×premixed reagent also contains Tli RNaseH, a heat-resistant RNase H, which minimizes inhibition of PCR due to residual mRNA when using cDNA as template. This product is suitable for high-speed PCR and allows accurate assay and detection of targets, making it possible to conduct real time PCR analyses with good reproducibility and high reliability.

Storage

2-8°C avoid light for 6 months, -20°C for two years. Do not freeze (Return to room temperature before use).

Components

1. SYBR Premix Ex Taq (2×Conc.) (5units,1.0 mL)
2. Specification：500preps.

DNA Preparation--*Please select ECALBIO of DNA Purification kit (Spin Column)*

-Blood

1. Take 500μL anticoagulant blood to a 1.5 mL clean centrifuge tube, add 1 ml of ddH_2O, shake for 30s .
2. Centrifuge at 8,000 rpm for 5 min, discard the supernatant.
3. Repeat washing until no red precipitate.
4. Add 1mL physiological saline, shake 15s, centrifuge at 10,000 rpm for 5 min, discard the supernatant.
5. Add 50μL DNA extraction and mix with precipitation.
6. 100℃ 10 min. Centrifuged at 13,000 rpm for 3min.
7. Take the supernatant 4μL for PCR reactions.

-Animal tissue, food or feed

1. Take about 30mg homogenized samples, and put into a 1.5mL clean centrifuge tube, add 100μL DNA extraction and mix well (the DNA extraction must dissolve at room temperature and fully mixed before use).
2. Water bath at 56℃ for 30 min, then at 100℃ for 10 min.
3. Centrifuge at 13,000rpm for 5 min.
4. Transfer the supernatant 5μL for PCR reactions.

Real Time PCR Reaction

1. Take out qPCR MIX from the kit, melt it at room temperature and oscillate it, then mix it with 10000 rpm centrifugal 10s;
2. Each of the 25μL system, the upstream and downstream primers were 0.5μL, add 12.5μL SYBR Green Premix and 9μL ddH_2O respectively, is up to 25μL system, mixed and amplificated.

PCR Solution	**PCR System** (20μL)	**PCR System** (25μL)
DNA Sample	2μL	2.5μL
Upstream primers	0.4μL	0.5μL
Downstream primers	0.4μL	0.5μL
2×SYBR Green Premix	10μL	12.5μL
ddH_2O	7.20μL	9.0μL

3. Example: IQTM5, ABI PRISM7500 and other instruments using thin walled tubes: circulation conditions: 95℃ for 3 minutes, then 95℃for 10-15s, 60℃ for30 s,72℃ for 30s, 40cycles. The user should set the corresponding amplification conditions according to their own primers, and the above conditions are for reference only.

PCR amplification		Step 1	95 ℃ for 3 min
	40 Cycle	step 2	94 ℃ for 10-15 s
			60 ℃ for 20 s
			72 ℃ for 30 s

Results determination

1. Under the test conditions established, Ct value ≤ 10, dilute theDNA Sample, the real time PCR test need to be done again.
2. 20 <Ct value ≤ 30, quantitative analysis is accuracy.
3. If 30 <Ct value ≤ 35, need to increase the volume of DNA sample , improve PCR amplification to get correct result.
4. Ct value>35, check amplification curve. If the amplification curve is logarithmic amplification curve, it was suspected positive, otherwise judged as negative.