

2 x Taqman PCR MasterMix(with UNG enzyme)

Item number: SR2111

Specification: 50T / 200T

Storage: -20°C Storage, after thawing should be thoroughly mixed before use to avoid a lot of bubbles.

Product Introduction: 2×TaqMan PCRmix (Probe qPCR) is a special reagent for Real Time PCR using probe method.

Taq DNA Polymerase, which contains antibody modification, can effectively inhibit the non-specific amplification caused by primer non-specific annealing or primer dimer at low temperature, and improve the specificity of amplification reaction. The reagent is specially formulated with an optimized qPCR Buffer, which greatly improves the amplification efficiency and detection sensitivity of the qPCR reaction. A good standard curve can be obtained in a wide quantification area for accurate quantification. The product contains UNG enzyme, which can prevent the aerosol contamination in the PCR environment. This reagent is compatible with fluorescent quantitative PCR instruments from many manufacturers, such as Applied Biosystems, Eppendorf, Bio-Rad and Roche.

Due to the high efficiency of qPCR for nucleic acid amplification, it is very easy to cause aerosol pollution such as pipette, table, PCR instrument, and air in qPCR experiments. When the experimental environment is polluted, it will lead to false positive results of negative samples. In this reagent, dTTP in dNTP is replaced by dUTP so that the amplified PCR product contains dU bases. In subsequent experiments, dU PCR products containing aerosol contamination will be degraded by UNG glycosylase in this reagent, thus eliminating the aerosol pollution effect. UNG enzyme in this reagent can eliminate up to 100,000 copies of nucleic acid pollutants when incubated at 50°C for 2 minutes. Therefore, this product is an ideal reagent for diagnostic quantitative PCR detection.

Product features:

1. Suitable for probe method Real Time qPCR reaction, can detect and quantify the target sequence.
2. When PCR reaction solution is prepared, only template, primer, probe and sterilized distilled water can be added to react, and the operation is simple and convenient.

Operation steps:

1. Prepare the PCR reaction system: Prepare the PCR mixture according to the following components (Prepare the reaction solution on the ice)

Components	20 μL	25 μL	50 μL	Final Conc.

2×Probe qPCR Mix	10 μL	12.5 μL	25 μL	1 x
Primer F (10μM)	0.2 to 0.8 μL	0.25 to 1 μL	0.5 to 2 μL	100-400nm
Primer R (10μM)	0.2 to 0.8 μL	0.25 to 1 μL	0.5 to 1 μL	100-400nm
Probe (10μM)	0.2 to 0.8 μL	0.25 to 1 μL	0.25 to 1 μL	100-400nm
Template	-	-	-	pg-ng
ddH ₂ O	To 20μL	To 25 μL	To 50 μL	-

2. PCR reaction procedure: Normally, the reaction can be performed using a two-step method; If the two-step amplification is not ideal, the PCR procedure can be set up by the three-step method.

Two-step real-time PCR

Anti-contamination step: 50°C for 2 minutes

Hot start: 95°C for 2 minutes

Denaturation: 95 ° C for 10 to 20 seconds }
Annealing/extension: 60°C 20~60 seconds } 40 to 45 cycles

Three-step real-time PCR

Anti-contamination step: 50°C for 2 minutes

Hot start: 95°C for 2 minutes

Denaturation: 95 ° C for 10 to 20 seconds }
Annealing: 56-64°C 10~30 seconds } 40~45 cycles
Extension: 72 ° C 10~60 seconds }

Please select the appropriate T_m according to the specific primer. Usually the primer T_m is designed around 60 °C.

Note:

1. Prepare the reaction reagent on the ice.
2. Avoid freezing and thawing the kit multiple times; Keep the kit at 4°C for frequent use.
3. Mix it upside-down and use it after slight centrifugation. If the reagent is not mixed well, its reactivity will be reduced.
4. real-time PCR amplifiers that require ROX reference dyes: ABI PRISM7000/770/7900HT, 7300/7500 Real-Time PCR System, 7500 Fast Real-Time PCR System, StepOne/StepOnePLUS, Stratagene Mx3000P, Mx3005P and other fluorescence quantitative PCR instrument, in the real-time PCR need to add ROX reference dye, to correct the fluorescence signal error generated between the holes of the instrument.