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DNA-qPCR Reaction Solution (UNG)

Cat. No. : CW3204T (1 mL)
CW3204M (5 mL)

Shipping and Storage : -20°C, if need frequent use, can be stored in 2-8°C, try to avoid repeated freeze-thaw.

Components

Component	CW3204T 1 mL	CW3204M 5 mL
DNA-qPCR Reaction Solution (UNG)	1 mL	5×1 mL
ddH_2O	1 mL	5×1 mL

Principle

CW3204 is a premixed system specially designed for real-time fluorescence quantitative PCR by probe method (TaqMan, Molecular Beacon, etc.) with a concentration of 2×, It contains new engineering DNA enzymes, PCR Buffer, dNTPs (dTTP is replaced by dUTP), UNG enzyme, Mg^{2+}, enhancers and stabilizers, and is easy to operate. It is mainly used to detect genomic DNA target sequence and cDNA target sequence after RNA reverse transcription.

This product contains highly sensitive engineering DNA enzyme, which can effectively reduce the non-specific amplification caused by the non-specific binding of primer and template or primer dimer during the reaction process, and greatly improve the detection sensitivity and amplification efficiency. The activation of the enzyme only needs to be incubated at 95°C for 3 min, greatly shortening the reaction time of PCR. The combination of optimized PCR buffer system and mixed enzyme can effectively inhibit the production of non-specific products, and can significantly improve the amplification efficiency of PCR, with stronger fluorescence signal and higher sensitivity.

Cautions

1. Please mix gently upside down before use, avoid foaming as far as possible, and use after a short centrifugation.
2. Avoid repeated freeze-thaw, which may degrade product performance. This product can be stored at -20°C for a long time. If you need to use frequently in the short term, it can be stored at 2-8°C.
3. ROX dye is used to correct the fluorescence signal error generated between holes of quantitative PCR instrument. This product does not contain ROX dye.

Procedure

The following examples are the conventional PCR reaction system and reaction conditions, which should be improved and optimized according to different template, primer structure and target fragment size in actual operation.

1. PCR reaction system (Suggested)

Reagent	25 μL Reaction system
DNA-qPCR Reaction Solution (UNG)	12.5 μL
Forward Primer, 10 μM	0.4-0.8 μL1)
Reverse Primer, 10 μM	0.4-0.8 μL
Probe2) 10 μM	0.4-0.8 μL
Template DNA3)	5 μL
ddH_2O	Up to 25 μL

Note:

1) Generally, a primer concentration of 0.2 μM can get better results, and 0.1-1.0 μM can be used as a reference for the set range. The concentration of primers can be increased when the amplification efficiency is not high. When non-specific reaction occurs, the concentration of primers can be reduced to optimize the reaction system.

2) The final concentration of the probe used is related to the fluorescence quantitative PCR instrument used, the type of probe and the type of fluorescent labeled substance. Please adjust the concentration according to the instructions of the instrument or the specific requirements for the use of each fluorescent probe during actual use.

3) The amount of DNA template is usually 10-100 ng genomic DNA or 1-10 ng cDNA as reference. Due to the different number of gene copies of the order contained in the template of different species, gradient dilution can be carried out on the template to determine the best use of template.

2. PCR Reaction Condition

Step	Dosage	Time
UNG enzyme digestion	37°C	2 min
predegeneration	95°C	3 min[1)]
denaturation	95°C	10 s[2)]
Annealing/extension	60°C	20 s[3)]

Note:

1) The enzyme used in this product can be activated under the condition of pre-denaturation at 95°C for 3 min. Under these conditions, most templates can be well unchained. For templates with high GC content and complex secondary structure, the predenaturation time can be extended to 5 minutes to make the initial template fully unchain. If the high-temperature treatment time is too long, the enzyme activity will be affected. For simple template, 1 min predenaturation can also be used, and the best predenaturation time can be determined according to the template situation.

2) It is recommended to adopt the two-step PCR reaction procedure, and the annealing temperature should be 58-64°C as the reference of the setting range. When the non-specific reaction occurs, the annealing temperature can be increased. If the primers with low Tm value are not good experimental results, the three-step PCR amplification can be attempted. Please set the annealing temperature in the range of 56°C to 64°C as the reference.

3) The annealing extension time of several common instruments is set in the following table: When using Roche, BioRad, Agilent, Macro Time, Dongshenglong and other companies' fluorescent quantitative PCR instrument, please set it at 20 s.

Please set it at 30 s when using ABI 7000/7300/7500.

The annealing/extension time can be set according to different types of instruments and different templates. Please follow the instructions of the instrument for experimental operation.