Cat.:MD2084-4-NA

AcuGenix™ Fast Direct RT-qPCR Master Mix with UNG (Low DNA)-V4 V01

Product Description

AcuGenix™ Fast Direct RT-qPCR Master Mix with UNG (Low DNA)-V4 is designed to perform PCR directly from samples without RNA extraction or sample preparation. This reagent consists of rapid DNA polymerase, Neoscript RTase, heat-labile uracil DNA glycosylase (TS-UNG), RNase Inhibitor, $MgCl_2$, dNTPs (with dUTP instead of dTTP), and stabilizers, for one-step reverse transcription and quantitative PCR (qRT-PCR). This reagent preforms high inhibitor-tolerance, and thus it can be directly applied to the detection of samples like throat swab, saliva, anti-coagulated whole blood, plasma, and serum without RNA extraction. This reagent uses special buffer for qPCR with mixed enzymes of anti-inhibitory DNA polymerase and TS-UNG. Therefore, it can obtain a good amplification of target genes in samples containing inhibitors and prevent false positive amplification caused by PCR residual and aerosol pollution. Through specialized treatment, this product is devoid of DNA residues and is appropriate for the development of biopharmaceutical-related detection reagents.

Components

1. 10×Fast Direct RTase/UNG Mix Ⅳ (NA)
2. 2×Fast Direct RT Premix Buffer Ⅳ (NA) (dUTP)

Storage

Store at -20±5℃. Vortex before use. Avoid freeze-thaw cycles.

Prepare Reaction Mix

Composition	Volume per Reaction	Volume per Reaction	Final Concentration
2×Fast Direct RT Premix Buffer Ⅳ (NA) (dUTP)	12.5 μL	25 μL	1×
10×Fast Direct RTase/UNG Mix Ⅳ (NA)	2.5 μL	5 μL	1×
25×Primer-Probe Mix [1]	1 μL	2 μL	1×
Sample [2]	——	——	——
ddH_2O	To 25 μL	To 50 μL	——

1. The final concentration of primers is usually 0.2 μM for better results. when the reaction performance is poor, the primer concentration can be adjusted within the range of 0.2-1 μM. The probe concentration is usually optimized in the range of 0.1-0.3 μM. Concentration gradient experiments can be performed to find the best combination of primers and probes.
2. Biological sample types vary in their inhibitor composition, inhibitor concentration, and target gene copy number. The effect of sample storage media (e.g., swab transport media) on amplification also differs. The optimal sample input volume should be determined according to sample type and required detection sensitivity. When necessary, samples may be pre-diluted with nuclease-free water or TE buffer. Recommended volumes for a 50 μL reaction: anticoagulated whole blood 2.5 μL (5%), plasma 10 μL (30%), serum 10 μL (30%), nasopharyngeal swab 10 μL (20%), saliva 10 μL (20%).

Reaction Programe

Standard PCR Protocol			
Procedure	Temp.	Time	Cycle
Reverse transcription	50℃	10-20 min	1
Hot-start	95℃	1-5 min	1
Denaturation	95℃	10-20 s	40-50
Annealing/ Extension	56-64℃	20-60 s	

FAST PCR Protocol			
Step	Temperature	Duration	Cycles
Reverse transcription	50℃	5 min	1
Hot-start	95℃	30 s	1
Denaturation	95℃	1-3 s	40-45
Annealing/ Extension	56-64℃	3-20 s	

*The temperature-sensitive (TS) UNG enzyme incorporated in this product exhibits activity at ambient temperature and undergoes inactivation during the reverse transcription procedure.

Quality Control

1. Functional testin: sensitivity, specificity, and reproducibility of qPCR.
2. No exogenous nuclease activity, no exogenous endo/exonuclease contamination.

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Technical Notes

1. The amplification rate of rapid DNA polymerase is no less than 1kb/10s. Different PCR instruments have different heating and cooling speeds, temperature control modes and thermal conductivity, thus optimization of your primer/probe concentration and running method in combination with your specific fast PCR instrument is essential.
2. The temperature for reverse transcription reaction is usually 50℃, and it can be optimized at 42-55℃. The time needed for reverse transcription can be optimized between 5-30 minutes.
3. If the Rn value of the PCR amplification is too low or the amplification is obviously inhibited, decreasing the sample amount, increasing reaction volume or previous dilution of the sample may improve the results.
4. The collection of blood, saliva, urine, throat swab, etc. should follow the clinical criteria requirements, and fresh sample can be used to prevent nucleic acid degradation.
5. The three-step PCR method is recommended for primers with low annealing temperature or for amplification of long fragments over 200 bp.
6. Since different amplicons have different utilization efficiency to dUTP and sensitivity to UNG, the reagents should be optimized if the detection sensitivity decreases when using UNG system. Please contact us for technical support if needed.
7. To avoid amplification of carryover PCR products between one-step reactions, dedicated experimental area and pipette are required for amplification. Operate with gloves and change frequently and do not open the reaction tube after PCR amplification.