Cat.:M2181-4

# AcuGenix™ FastAmpli All-in-One qPCR Master Mix with UNG-V4

V01

## Product Description

AcuGenix™ FastAmpli All-in-One qPCR Master Mix with UNG-V4 is a probe-based reagent specifically formulated for qualitative and quantitative Real-Time PCR applications. It incorporates a genetically engineered fast-amplification DNA polymerase capable of completing PCR amplification within 30 min. The buffer system has been optimized for multiplex amplification. This reagent contains an anti-inhibitory amplification enzyme and UNG enzyme mixture combined with an optimized dUTP-containing buffer system, enabling robust amplification of target genes in inhibitor-containing samples while effectively preventing false-positive results caused by PCR product carry-over and aerosol contamination. This reagent is compatible with most real-time PCR instruments from major manufacturers, including Applied Biosystems, Eppendorf, Bio-Rad, and Roche.

## Components

2×AcuGenix™ FastAmpli All-in-One qPCR Master Mix with UNG-V4

*This reagent already contains hot-start DNA polymerase, UNG, PCR Buffer, $MgCl_2$, dNTPs, and stabilizers.

## Storage

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

## Prepare Reaction Mix

| Components | 25 μL System | 50 μL System | Final Concentration |
|---|---|---|---|
| 2×AcuGenix™ FastAmpli All-in-One qPCR Master Mix with UNG-V4 | 12.5 μL | 25 μL | 1× |
| 25×Primer-Probe Mix [1,2] | 1 μL | 2 μL | 1× |
| Template DNA [3] | — | — | — |
| $ddH_2O$ | To 25 μL | To 50 μL | — |

1. When using the rapid PCR protocol, appropriately increasing primer and probe concentrations may yield improved amplification results. The primer-to-probe ratio should be optimized empirically.
2. When using the standard PCR protocol, a final primer concentration of 0.2 μM typically yields optimal results. If assay performance is suboptimal, primer concentrations may be adjusted within the range of 0.2-1 μM. Probe concentrations are typically optimized within the range of 0.1-0.3 μM. Gradient titration experiments are recommended to determine the optimal primer-probe combination.
3. Different template types contain different copy numbers of the target gene; gradient dilutions may be performed as necessary to determine the optimal template quantity.

## Reaction Programe

| Standard PCR Protocol | | | |
|---|---|---|---|
| Procedure | Temp | Time | Cycle |
| UNG treatment | 50°C | 2 min | 1 |
| Hot-start | 95°C | 1-5 min | 1 |
| Denaturation | 95°C | 10-20 s | 40-50 |
| Annealing/Extension | 56-64°C | 20-60 s | |

| FAST PCR Protocol | | | |
|---|---|---|---|
| Procedure | Temp | Time | Cycle |
| UNG treatment | 50°C | 2 min | 1 |
| Hot-start | 95°C | 30 s | 1 |
| Denaturation | 95°C | 1-3 s | 40-45 |
| Annealing/Extension | 56-64°C | 3-20 s | |

## Quality Control

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

## Notes

1. The fast-amplification DNA polymerase incorporated in this reagent has an extension rate of no less than 1 kb/10 s. Different PCR instruments exhibit significant differences in temperature ramp rate, temperature control mode, and thermal conductivity. Therefore, it is recommended to optimize the optimal reaction conditions based on the specific characteristics of the fast PCR instrument.
2. This reagent exhibits enhanced specificity, significantly improving the detection sensitivity of real-time PCR at the limit of detection. It markedly improves amplification curve uniformity and fluorescence signal intensity at extremely low

template concentrations, making it suitable as a high-sensitivity real-time PCR detection reagent.

3. For primers with low annealing temperatures or amplicon fragments longer than 200 bp, it is recommended to use a three-step method for amplification.

4. The efficiency of dUTP utilization and sensitivity to UNG enzyme varies for different genes to be amplified. If the detection sensitivity is reduced due to the use of the UNG system, adjust and optimize the reaction system accordingly. If technical support is required, please contact our company.

5. Use dedicated areas and pipettes before and after amplification, wear gloves during operation, and regularly replace them. Do not open reaction tubes after PCR completion to minimize sample contamination from PCR products.