# ® •ZmTech Universal qPCR Mixtures (Cat. K209080)

## Kit contains:

Stored at -20°C

- 2x Ready-to-use universal qPCR mixture, including optimization of 2x PCR Buffer, Heat-Activated Taq DNA Polymerase, dNTPs(dATP, dCTP, dGTP, dTTP), 6mM $MgCl_2$, PCR Buffer Stabilizer, RNase/DNase I Inhibitors, Internal Reference and Green I Dye Chemistry.

## Reagents required but not provided:

- PCR grade water and Reverse Transcriptase (RT).

## Description:

- ZmTech universal qPCR mixture (K209080) is a 2x reaction mixture suitable for most real-time PCR assays, designed for qPCR assays where template is limited or very low-copy-number targets. It has been developed to minimize primer-dimers, which are of particular concern when using dye chemistry for detection.
- ZmTech universal qPCR mixture contains low levels of internal reference, ideal for those block-based platforms which are more sensitive to dye levels. This internal reference dye is necessary for ABI Sequence detection systems, and no interfere with LightCyler I/II systems.
- Suggestions for use: The Taq DNA polymerase is inactive at room temperature and a 5-minute hot start at 95$^{o}$C is essential to active the enzyme. Hot start procedure can significantly improve the results of DNA amplifications by reducing the generation of nonspecific amplification products and primer-dimer artifacts.

## Reaction conditions/suggestions for use:

- RNA preparation: Integrity and Purity of RNA template is essential. Impurities (proteins, phenol, chloroform) may interfere the RT-PCR Reactions and detection systems.
- $MgCl_2$ Optimization: $MgCl_2$ is a co-factor for the Taq DNA Polymerase. A final $MgCl_2$ concentration of 3mM should be ideal for most applications. However, the $MgCl_2$ requirements for the polymerase often vary, depending on the particular template and primers used. $MgCl_2$ can be optimized within 3mM-7mM range.
- Primer Concentration: A final primer concentration of 200nM is sufficient for most reactions. However, primer concentration can be optimized within 25-900nM range.
- cDNA Template Concentration: 50ng of human cDNA in a 25ul reaction is ideal for real-time PCR reactions. However, this may vary, depending on the particular gene of interest. Too high template concentration will inhibit PCR reactions.

## Procedure: (suggested protocol)

1. Preparation of PCR Master Mix

for a single reaction (total volume: 25uL) in a PCR microtube or plate

| Component | Volume (μL) | Final Concentration |
|---|---|---|
| All-IN-One qPCR Mixture(2x) | 12.5 | 1x |
| $MgCl_2$ | X | (3mM-7mM) |
| DNA Template | 2 | (100pg-500pg/μL) |
| Forward primer (5μM) | 1 | 200nM |
| Reverse primer (5μM) | 1 | 200nM |
| PCR grade water | up to 25 μL | |

## 2. Setup typical thermal cycling parameters

| | | |
|---|---|---|
| Enzyme activation Step: | 95°C | 5 minutes |
| 25-40 Cycles: | | |
| Denaturation | 95°C | 15 seconds |
| Annealing | X°C | 30 seconds dependent on Tm of primers |
| Extension | 60°C | 30 seconds (acquire data at the end of this step) |

Or: Run reactions in a 25ul glass capillary tube.

| Step: | Temperature: | Time: | Temperature Transition Rate |
|---|---|---|---|
| 1. Denaturation (1 cycle) | 94°C | 5 minutes | 20°C/second |
| 2. Cycles (35 cycles): | | | |
| Denature | 94°C | 15 seconds (none) | 20°C/second |
| Annealing | X°C | 15 seconds (none) | 20°C/second |
| Extension | 72°C | 8 seconds (none) | 20°C/second |
| Detection | 72°C | 0 seconds (single) | 20°C/second |
| 3. Melting(1 cycle) | X°C | 1 minute (none) | 20°C/second |
| | | (95°C, 0 Second, | 0.15°C/second, con.) |
| 4. Cooling (1cycle) | 40°C | 30 seconds (none) | 20°C/second |

Precautions and Disclaimer:

- This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.