® •ZmTech Universal qPCR Mixtures (Cat. K209090)

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 and Internal Reference.**

Reagents required but not provided:

- **PCR grade water and Reverse Transcriptase (RT).**

Description:

- **ZmTech universal qPCR mixture (K209090) 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 fluorescent probe 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°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.**
- **Probe Concentration: In most case, using a combination of primers and probe at a molar ratio of 3:1 is sufficient. However, probe concentration can be optimized within the 10-250nM range.**

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
Probe	1	70nM
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.