Product Information

ZmTech® Improved Fluo-Probe qPCR Mixture

Catalog Number: **A207020**
Storage Temperature: **-20°C**

Kit contains:
An Improved qPCR Mixture (2x), containing 2x PCR reaction Buffer, Heat-Activated Taq DNA Polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), 6mM $MgCl_2$, PCR Buffer Stabilizers, RNase/DNase I inhibitors, and Internal reference.

Description:
ZmTech® Improved quantitative PCR Mixture is a 2x reaction mix designed for the quantitative real-time PCR 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® Improved qPCR Mixture contains low levels of internal reference dye, 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 10-minute hot start at 95ºC 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.

Storage and stability:
Store this kit at -20°C in a constant-temperature freezer. Avoid prolonged exposure to light.

Reaction conditions/suggestions for use:

- RNA preparation: Integrity and Purity of RNA template is essential. Impurities (proteins, phenol, chloroform) may interfere the qPCR 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.
- cDNA template concentration: An excess of template will inhibit PCR reactions. 100ng of cDNA template in a 25ul reaction is normally sufficient. However , this may vary, depending on the particular gene of interest.

Procedure:

I. Preparation of PCR Master Mix for a single reaction (total volume: 25uL) in a 0.2 ml or 0.5ml microtube

Component	Volume (µL)	Final Concentration
1. Improved qPCR Mixture (2X)	12.5	(1x)
2. $MgCl_2$	*	(3mM-7mM)
3. DNA Template	2-5	(determined by user)
4. Froward Primer (5µM)	1	200nM
5. Reverse Primer (5µM)	1	200nM
6. Probe	1	70nM
7. PCR grade water	*	up to 25 µL

II. Setup typical thermal cycling parameters

1. Enzyme activation Step:	95°C	10 minutes
2. 25-40 Cycles:		
Denaturation	95°C	15 seconds
Annealing	X°C	30 seconds
	Dependent on Tm of primers	
Extension	72°C	(acquire data)
		60 base pairs/second

III. Troubleshooting

	Possible Cause	Recommendation
1. Low/no Signal	.Too few cycles	Increase cycles
	.Too high annealing temperature	Decrease temperature in increments of 2-4°C
	.Poor template	Increase template con.
	.Primers degraded	Redesign/optimization
2. Multiple Products	.Too high template	Decrease template con.
	.Too high primer	Decrease primer con. in 2 fold dilutions
	.Too low annealing temperature	Increase temperature in increments of 2-3°C
	. Primer-dimers	Hot-start 95°C 10mins
3. No linear standard curve	.Too high or low template	Decrease or increase template concentration
	. Primer-dimers	Hot-start 95°C 10mins
4. Fluo-rescence variable	.Too high or low template	Decrease or increase template concentration
	.qPCR instrument contaminated	Decontaminate qPCR instrument
	. No well mix	Mix qPCR mixture well

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.