vivantis

ViPrimePLUSHigh 4X One Step RT-qPCR Green Master Mix I (SYBR® Green Dye)

Product code:	QLMM10
Pack size:	100 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUSHigh 4X One Step RT-qPCR Green Master Mix I is the improved version of master mix working for one-step amplification of any RNA template including total RNA, mRNA and etc using real-time PCR method.

ViPrimePLUSHigh 4X One Step RT-qPCR Green Master Mix I is improved to have 4X concentration and consists of improved technology of enzyme of Reverse Transcriptase and Hot Start *Taq* DNA Polymerases; dNTPs/dUTP Mix, RNase Inhibitor and Heat-labile UDG that allow less contaminated reaction preparation as well as leads to increased performance in sensitivity and specificity. This master mix is having SYBR® Green dye emits fluorescence when bound to double-stranded DNA without probes. Detection of PCR product is monitored by the increase in fluorescence.

Separated tubes of ROX™ dye are provided to allow the master mixes to use with most of the real-time PCR instruments. The master mixes can be used for fast cycling as suggested for any qualitative tests.

APPLICATIONS

All kinds of RNA sample material suited for RT-qPCR amplification can be used.

FEATURES

- One step reaction set up for real-time PCR or conventional PCR
- Equipped with improved generation of thermostable Reverse Transcriptase and Hot Start *Taq* DNA Polymerases
- Includes SYBR® Green dye for intercalator-based qPCR
- Optimal performance for highly sensitive and specific RT-PCR reaction compared to QLMM04
- Compatible with most of the real-time PCR platforms

COMPONENTS

- 1ml Nuclease-free Water
- 0.5ml ViPrimePLUSHigh 4X One Step RT-qPCR Green Master Mix I
- 0.5ml LOW ROX™ Dye
- 0.5ml HIGH ROX™ Dye

STORAGE

Stable at -20°C up to the expiry date stated. Store all components at -20°C upon arrival. Keep in aliquot to reduce freeze-thaw cycles.

QUALITY CONTROL

As part of the ISO9001:2015 quality assurance systems, each lot of ViPrimePLUSHigh 4X One Step RT-qPCR Master Mix has been tested against pre-determined specifications to ensure consistent product quality and highest levels of performance and reliability.

LIMITATION OF USE

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

INSTRUMENTS

To calibrate a real-time PCR reaction, various formulations of master mixes are available for most of the platforms.

Master Mixes with Compatible Hardware
Original: No ROX added
Analytik Jena qTower series, BioRad iCycler all series, BioRad CFX96 & CFX384, Cepheid SmartCycler®, Eppendorf Mastercycler series, Fluidigm BioMark™, Illumina Eco, MJ Chromo4, Opticon, PCRMax Eco™, Roche lightcycler® series, Qiagen RotorGene, Thermo PikoReal™
LOW ROX™ Dye added into master mix
Agilent / Stratagene MX MX3000P®, MX3005P®, MX4000®, Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
HIGH ROX™ Dye added into master mix
Applied Biosystems 7000,7300,7700,7900 and 7900HT FAST platforms, OpenArray PRISM 7000,7700,7900, GeneAmp® 5700, StepOne™, StepOne™ PLUS

PROTOCOL

1. Keep the RT-qPCR master mix protected from light before and after use.
2. Aliquot the RT-qPCR master mix to minimize freeze-thaw cycles and light exposure.
3. Mix well the master mix before use as glycerol ingredient in master mix.
4. When preparing mixes, always calculate the volume according to the number of reactions that needed plus one extra.

SUGGESTED MIXTURE

When using user's supplied primers and probe:
Check the hardware platform to add type of ROX dye before performing test.

Components	Reaction (1X)	Reaction using LOW ROX (1X)	Reaction using HIGH ROX (1X)
ViPrimePLUSHigh 4X One Step RT-qPCR Green Master Mix I	5.0 µl	5.0 µl	5.0 µl
Low ROX™ Dye / High ROX™ Dye	0.0 µl	5.0 µl	5.0 µl
Primers (10µM Forward & Reverse)	0.4 µl	0.4 µl	0.4 µl
Template	1 pg – 1 µg	1 pg – 1 µg	1 pg – 1 µg
Nuclease free water	Top up to 20 µl	Top up to 20 µl	Top up to 20 µl
Final Volume	20.0 µl	20.0 µl	20.0 µl

CYCLING PROGRAM

a. Standard cycling

Step	Cycles	Temp	Time
Reserve Transcription	1	55°C	15mins
Enzyme activation	1	95°C	30secs
Denaturation	45**	95°C	10secs
Data Collection*		60°C	30secs
Melt Curve***			

*Fluorogenic data should be collected during this step through the FAM channel. Annealing time can be adjusted for different platforms up to 40-60 secs for better performance if needed to.
**A further 10 cycles can be added to generate the complete amplification plot for low copy number targets which giving late detection.
***A post PCR run melt curve can be used to prove the specificity of primers.

b. Fast cycling

Step	Cycles	Temp	Time
Reserve Transcription	1	55°C	5mins
Enzyme activation	1	95°C	30secs
Denaturation	45	95°C	5secs
Data Collection		60°C	20secs

Fast cycling can be suggested for qualitative test. To have optimization, standard cycling as suggested is recommended to use before go into fast cycling.

PREVENTION OF CONTAMINATION

Extra care should be taken to eliminate the possibility of contamination with any foreign RNA templates.

- Use separate clean areas for preparation of samples, reaction mixture and for cycling.
- Clean lab bench and equipments periodically with 3% hydrogen peroxide or 70% ethanol.
- Wear fresh gloves. Change gloves whenever suspect that they are contaminated.
- Use sterile tubes and pipette tips with aerosol filters for PCR reaction set up.
- With every PCR reaction set up, perform a contamination control reaction without template RNA.

TROUBLESHOOTING

Possibility	Suggestion
Problem: Negative control / no template control gives positive result	
1. Carry over contamination	Change nuclease-free water. Use fresh aliquots of reagents. Use filtered tips. Load positive control last.
Problem: No signal detected	
1. Incorrect programming of instrument	Check program.
2. Reagents expired	Check the expiry date of reagents before repeat.
3. Storage condition not complying with instructions	Check storage condition properly and store at correct storage condition to avoid the degradation of reagents.
Problem: Early / late signal detected than expected	
1. Genomic DNA/RNA contamination or multiple products	DNase or RNase treatment of template before qPCR; re-design primers to increase specificity
2. Unspecific products or primer dimers detected	Re-design primers to increase specificity
3. Limiting reagents or degraded reagents such as master mix	Check calculations for master mix; repeat experiment using fresh stock solutions
4. Poor efficiency during PCR reaction	Re-design primers to a different region of the target sequence
5. Unanticipated variants within target sequence	Keep the GC content to between 30-50%

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Purchase of product does not include a license to perform any patented applications; therefore it is the sole responsibility of users to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used.

WARRANTY AND LIMITED LIABILITY
The performance characteristics stated were obtained using the assay procedures in the insert. Failure to comply with the instructions may derive inaccurate results. In such event, manufacturer disclaims all warranty expressed, implied or statutory including the implied warranty of merchantability and the fitness of use.

The manufacturer will not be liable for any damage caused by misuse, improper handling and storage; non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.

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