ViPrimePLUS At*Taq* qPCR Green Master Mix II with Low ROX (EvaGreen® Dye)

Product code:	QLMM07-LR
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS At*Taq* qPCR Green Master Mix II is next generation first choice mix designed for fast and easy real-time PCR reaction set up. The improved formulation of master mix contains pure Hot Start *Taq* DNA Polymerases, EvaGreen® dye, ROX™ dye, highest quality dNTPs, and buffer components at optimal concentrations. Hot Start *Taq* DNA Polymerases in the master mix provide antibody mediated hot start mechanism which releases more active enzyme and requires shorter activation time. EvaGreen® dye in master mix is environmentally safe and highly stable which can be formulated with relative high dye concentration to maximize fluorescence signal without PCR inhibition.

ViPrimePLUS At*Taq* qPCR Green Master Mix II can be used to amplify any DNA template including genomic, cDNA and viral sequences. The improved formulation of qPCR green master mix can detect extremely low copy number targets very specifically with high efficiency. The qPCR green master mix is designed to prevent and reduce the formation of primer dimers and non-specific products leading to optimum sensitivity and specificity.

ViPrimePLUS At*Taq* qPCR Green Master Mix II has several formulations optimized to be used with most of real-time PCR instruments. The improved sensitivity and consistency of ViPrimePLUS At*Taq* qPCR Green Master Mix II in standard cycling conditions gives the industry leading performance in fast cycling conditions.

APPLICATIONS

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

FEATURES

- Fast and easy real-time PCR reaction set up
- Rapid extension rate for early Ct values
- Contain Hot Start *Taq* DNA Polymerase - highest sensitivity and specificity
- Contain EvaGreen® dye – highest dye stability and safety
- Increased limit of detection
- Minimal PCR inhibition
- Compatible with most of the real-time PCR platforms

COMPONENTS

1.6ml aliquots of master mix

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 ViPrimePLUS At*Taq* qPCR Green Master Mix II has been tested against predetermined 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	
QLMM07	ViPrimePLUS At*Taq* qPCR Green Master Mix II (EvaGreen® Dye) 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™
QLMM07-LR	ViPrimePLUS At*Taq* qPCR Green Master Mix II with Low ROX (EvaGreen® Dye) Agilent / Stratagene MX MX3000P®, MX3005P®, MX4000®, Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM07-R	ViPrimePLUS At*Taq* qPCR Green Master Mix II with ROX (EvaGreen® Dye) Applied Biosystems 7000,7300,7700,7900 and 7900HT FAST platforms, OpenArray PRISM 7000,7700,7900, GeneAmp® 5700, StepOne™, StepOne™ PLUS

PROTOCOL

1. Keep the qPCR green master mix protected from light before and after use.
2. Aliquot the qPCR green master mix to minimize freeze-thaw cycles and light exposure.
3. Reserve plate positions for positive (control DNA) and negative (water or buffer) controls.
4. When preparing mixes, always calculate the volume according to the number of reactions that needed plus one extra.
5. After the mixture is prepared and aliquoted into tubes, place them into qPCR platform.

SUGGESTED MIXTURE

a. When using ViPrimePLUS gene detection kits:

Components	Reaction (1X)
At*Taq* qPCR Green Master Mix II	10µl
Primer/Probe Mix	1µl
Template (25ng)	5µl
Nuclease free water	4µl
Final Volume	20µl

b. When using user's supplied primers and probe:

Components	Reaction (1X)
At*Taq* qPCR Green Master Mix II	10µl
Primers (6pmols Forward & Reverse)	X µl
Probe (3pmols)	X µl
Template (25ng)	X µl
Nuclease free water	X µl
Final Volume	20µl

CYCLING PROGRAM

a. For Taqman® gene detection kits

Step	Cycles	Temp	Time
Enzyme activation	1	95°C	2mins
Denaturation	40**	95°C	15secs
Data Collection*		60°C	60secs

*Fluorogenic data should be collected during this step through the FAM channel.

**A further 10 cycles can be added to generate the complete amplification plot for low copy number targets which giving late detection.

b. For EvaGreen® detection kits

Step	Cycles	Temp	Time
Enzyme activation	1	95°C	2mins
Denaturation	40***	95°C	15secs
Data Collection*		60°C	60secs
Melt Curve**			

*Fluorogenic data should be collected during this step through the EvaGreen® channel.

**A post PCR run melt curve can be used to prove the specificity of primers. See the manufactures instructions for your hardware platform.

***A further 10 cycles can be added to generate the complete amplification plot for low copy number targets which giving late detection.

PREVENTION OF CONTAMINATION

qPCR amplification is a very sensitive DNA amplification reaction; therefore extra care should be taken to eliminate the possibility of contamination with any foreign DNA 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 DNA.

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%

LEGAL DISCLAIMER

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.