vivantis

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix

Product code:	QLMM03
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix is next generation first choice mix designed for fast and easy one step real-time PCR reaction set up. The improved formulation of master mix contains unique thermostable M-MULV enzyme, Hot Start *Taq* DNA Polymerases as well as $MgCl_2$ and buffer components at optimal concentrations. The M-MULV enzyme has an optimal operating temperature of 55°C, and has a higher affinity for primer template duplexes which allows very rapid processing during RT step. The One Step At*Taq* RT-qPCR Master Mix is designed to achieve excellent results in reaction efficiency, correlation coefficient and slope.

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix can be used to amplify any RNA template including mRNA, total RNA and viral sequences. The improved formulation of RT-qPCR master mix can detect extremely low copy number targets very specifically with high efficiency that gives CT values close to the theoretical time of detection. The ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix is a complete system for use in one step real-time PCR, the removal of a separate reverse transcription step reduces handling errors as well as the time taken to obtain results. The formation of primer dimers and non-specific products is prevented leading to optimum sensitivity and specificity.

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

APPLICATIONS

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

FEATURES

- One step real time RT-qPCR reaction set up
- Equipped with thermostable M-MULV enzyme and Hot Start *Taq* DNA Polymerases
- Reliable quantification of extremely low copy number targets
- Optimal performance for highly sensitive and specific RT-qPCR reaction
- Compatible with most of the real-time PCR platforms

COMPONENTS

3 x 0.6ml aliquots of master mix
0.6ml aliquots of “no RT control master mix standard”

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 ISO9001:2008 quality assurance systems, each lot of ViPrimePLUS One Step At*Taq* 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	
QLMM03	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix Biometra qTower, BioRad iCycler, BioRad IQ4, BioRad IQ5, Cepheid SmartCycler®, Eppendorf Mastercycler, Fluidigm BioMark™, Illumina Eco, MJ Chromo4, Opticon, PCRMax Eco™, Roche lightcycler® 480, lightcycler® LC96 and lightcycler® Nano Platforms, RotorGene, Roche Capillary Lightcycler 1.0-2.0, Stratagene MX MX4000P®, MX3000P®, MX3005®, Thermo PikoReal™
QLMM03-LR	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with Low ROX Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM03-R	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with ROX Applied Biosystems 7000, 7300, 7700, 7900 and 7900HT FAST platforms, 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. Reserve plate positions for positive (control RNA) 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 RT-qPCR platform.

SUGGESTED MIXTURE

a. When using ViPrimePLUS gene detection kits:

Components	Reaction (1X)
At*Taq* One Step RT-qPCR Master Mix	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* One Step RT-qPCR Master Mix	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
Reserve Transcription	1	55°C	10mins
Enzyme activation	1	95°C	8mins
Denaturation	40**	95°C	10secs
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 SYBR® Green detection kits

Step	Cycles	Temp	Time
Reverse Transcription	1	55°C	10mins
Enzyme activation	1	95°C	8mins
Denaturation	40***	95°C	10secs
Data Collection*		60°C	60secs
Melt Curve**			

*Fluorogenic data should be collected during this step through the SYBR® Green 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

RT-qPCR amplification is a very sensitive RNA amplification reaction; therefore 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%

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.

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ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with Low ROX

Product code:	QLMM03-LR
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix is next generation first choice mix designed for fast and easy one step real-time PCR reaction set up. The improved formulation of master mix contains unique thermostable M-MULV enzyme, Hot Start *Taq* DNA Polymerases, ROX dye as well as $MgCl_2$ and buffer components at optimal concentrations. The M-MULV enzyme has an optimal operating temperature of 55°C, and has a higher affinity for primer template duplexes which allows very rapid processing during RT step. The One Step At*Taq* RT-qPCR Master Mix is designed to achieve excellent results in reaction efficiency, correlation coefficient and slope.

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix can be used to amplify any RNA template including mRNA, total RNA and viral sequences. The improved formulation of RT-qPCR master mix can detect extremely low copy number targets very specifically with high efficiency that gives CT values close to the theoretical time of detection. The ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix is a complete system for use in one step real-time PCR, the removal of a separate reverse transcription step reduces handling errors as well as the time taken to obtain results. The formation of primer dimers and non-specific products is prevented leading to optimum sensitivity and specificity.

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

APPLICATIONS

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

FEATURES

- One step real time RT-qPCR reaction set up
- Equipped with thermostable M-MULV enzyme and Hot Start *Taq* DNA Polymerases
- Reliable quantification of extremely low copy number targets
- Optimal performance for highly sensitive and specific RT-qPCR reaction
- Compatible with most of the real-time PCR platforms

COMPONENTS

3 x 0.6ml aliquots of master mix
0.6ml aliquots of “no RT control master mix standard”

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 ISO9001:2008 quality assurance systems, each lot of ViPrimePLUS One Step At*Taq* 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	
QLMM03	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix Biometra qTower, BioRad iCycler, BioRad IQ4, BioRad IQ5, Cepheid SmartCycler®, Eppendorf Mastercycler, Fluidigm BioMark™, Illumina Eco, MJ Chromo4, Opticon, PCRMax Eco™, Roche lightcycler® 480, lightcycler® LC96 and lightcycler® Nano Platforms, RotorGene, Roche Capillary Lightcycler 1.0-2.0, Stratagene MX MX4000P®, MX3000P®, MX3005®, Thermo PikoReal™
QLMM03-LR	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with Low ROX Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM03-R	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with ROX Applied Biosystems 7000, 7300, 7700, 7900 and 7900HT FAST platforms, 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. Reserve plate positions for positive (control RNA) 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 RT-qPCR platform.

SUGGESTED MIXTURE

a. When using ViPrimePLUS gene detection kits:

Components	Reaction (1X)
At*Taq* One Step RT-qPCR Master Mix	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* One Step RT-QPCR Master Mix	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
Reserve Transcription	1	55°C	10mins
Enzyme activation	1	95°C	8mins
Denaturation	40**	95°C	10secs
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 SYBR® Green detection kits

Step	Cycles	Temp	Time
Reverse Transcription	1	55°C	10mins
Enzyme activation	1	95°C	8mins
Denaturation	40***	95°C	10secs
Data Collection*		60°C	60secs
Melt Curve**			

*Fluorogenic data should be collected during this step through the SYBR® Green 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

RT-qPCR amplification is a very sensitive RNA amplification reaction; therefore 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%

LEGAL DISCLAIMER

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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.

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with ROX

Product code:	QLMM03-R
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix is next generation first choice mix designed for fast and easy one step real-time PCR reaction set up. The improved formulation of master mix contains unique thermostable M-MULV enzyme, Hot Start *Taq* DNA Polymerases, ROX dye as well as $MgCl_2$ and buffer components at optimal concentrations. The M-MULV enzyme has an optimal operating temperature of 55°C, and has a higher affinity for primer template duplexes which allows very rapid processing during RT step. The One Step At*Taq* RT-qPCR Master Mix is designed to achieve excellent results in reaction efficiency, correlation coefficient and slope.

ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix can be used to amplify any RNA template including mRNA, total RNA and viral sequences. The improved formulation of RT-qPCR master mix can detect extremely low copy number targets very specifically with high efficiency that gives CT values close to the theoretical time of detection. The ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix is a complete system for use in one step real-time PCR; the removal of a separate reverse transcription step reduces handling errors as well as the time taken to obtain results. The formation of primer dimers and non-specific products is prevented leading to optimum sensitivity and specificity.

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

APPLICATIONS

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

FEATURES

- One step real time RT-qPCR reaction set up
- Equipped with thermostable M-MULV enzyme and Hot Start *Taq* DNA Polymerases
- Reliable quantification of extremely low copy number targets
- Optimal performance for highly sensitive and specific RT-qPCR reaction
- Compatible with most of the real-time PCR platforms

COMPONENTS

3 x 0.6ml aliquots of master mix
0.6ml aliquots of “no RT control master mix standard”

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 ISO9001:2008 quality assurance systems, each lot of ViPrimePLUS One Step At*Taq* 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	
QLMM03	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix Biometra qTower, BioRad iCycler, BioRad IQ4, BioRad IQ5, Cepheid SmartCycler®, Eppendorf Mastercycler, Fluidigm BioMark™, Illumina Eco, MJ Chromo4, Opticon, PCRMax Eco™, Roche lightcycler® 480, lightcycler® LC96 and lightcycler® Nano Platforms, RotorGene, Roche Capillary Lightcycler 1.0-2.0, Stratagene MX MX4000P®, MX3000P®, MX3005®, Thermo PikoReal™
QLMM03-LR	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with Low ROX Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM03-R	ViPrimePLUS One Step At*Taq* RT-qPCR Master Mix with ROX Applied Biosystems 7000, 7300, 7700, 7900 and 7900HT FAST platforms, 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. Reserve plate positions for positive (control RNA) 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 RT-qPCR platform.

SUGGESTED MIXTURE

a. When using ViPrimePLUS gene detection kits:

Components	Reaction (1X)
At*Taq* One Step RT-qPCR Master Mix	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* One Step RT-QPCR Master Mix	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
Reserve Transcription	1	55°C	10mins
Enzyme activation	1	95°C	8mins
Denaturation	40**	95°C	10secs
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 SYBR® Green detection kits

Step	Cycles	Temp	Time
Reverse Transcription	1	55°C	10mins
Enzyme activation	1	95°C	8mins
Denaturation	40***	95°C	10secs
Data Collection*		60°C	60secs
Melt Curve**			

*Fluorogenic data should be collected during this step through the SYBR® Green 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

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- 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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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.