ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I (SYBR® Green Dye)

Product code:	QLMM14
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I is next generation master mix designed for one step real-time PCR reaction set up. The master mix is prepared in 2X concentrated solution and contains unique thermostable M-MULV enzyme, *Taq* DNA Polymerases, SYBR® Green dye as well as $MgCl_2$ and buffer components at optimal concentrations. The M-MULV enzyme has an optimal operating temperature and a higher affinity for primer template duplexes which allows very rapid processing during RT step. SYBR® Green dye emits fluorescence when bound to double-stranded DNA. Detection of PCR product is monitored by the increase in fluorescence, leading to high sensitivity, wide dynamic range and high reproducibility for quantification.

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I can be used to amplify any RNA template including mRNA, total RNA and viral sequences. The formulation of RT-qPCR master mix can detect low copy number targets very specifically with high efficiency that give CT values close to the theoretical time of detection. The ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I 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 master mix provides convenient and robust set up for quantitative real-time analysis of RNA samples.

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I has several formulations optimized to be used with most of real-time PCR instruments. The sensitivity and consistency of ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I 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 SYBR® Green dye for intercalator-based qPCR
- Good buffer system for excellent amplification efficiency
- High sensitivity detection
- 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 *Taq* RT-qPCR Green Master Mix I 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	
QLMM14	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I (SYBR® Green Dye) 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™
QLMM14-LR	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with Low ROX (SYBR® Green Dye) Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM14-R	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with ROX (SYBR® Green Dye) 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)
Taq One Step RT-qPCR Green Master Mix I	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)
Taq One Step RT-qPCR Green Master Mix I	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 *Taq* RT-qPCR Green Master Mix I with Low ROX (SYBR® Green Dye)

Product code:	QLMM14-LR
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I is next generation master mix designed for one step real-time PCR reaction set up. The master mix is prepared in 2X concentrated solution and contains unique thermostable M-MULV enzyme, *Taq* DNA Polymerases, SYBR® Green dye, ROX dye as well as $MgCl_2$ and buffer components at optimal concentrations. The M-MULV enzyme has an optimal operating temperature and a higher affinity for primer template duplexes which allows very rapid processing during RT step. SYBR® Green dye emits fluorescence when bound to double-stranded DNA. Detection of PCR product is monitored by the increase in fluorescence, leading to high sensitivity, wide dynamic range and high reproducibility for quantification.

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I can be used to amplify any RNA template including mRNA, total RNA and viral sequences. The formulation of RT-qPCR master mix can detect low copy number targets very specifically with high efficiency that give CT values close to the theoretical time of detection. The ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I 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 master mix provides convenient and robust set up for quantitative real-time analysis of RNA samples.

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I has several formulations optimized to be used with most of real-time PCR instruments. The sensitivity and consistency of ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I 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 SYBR® Green dye for intercalator-based qPCR
- Good buffer system for excellent amplification efficiency
- High sensitivity detection
- 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 *Taq* RT-qPCR Green Master Mix I 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	
QLMM14	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I (SYBR® Green Dye) 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™
QLMM14-LR	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with Low ROX (SYBR® Green Dye) Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM14-R	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with ROX (SYBR® Green Dye) 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)
Taq One Step RT-qPCR Green Master Mix I	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)
Taq One Step RT-qPCR Green Master Mix I	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

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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.

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with ROX (SYBR® Green Dye)

Product code:	QLMM14-R
Packsize:	150 reactions
Lot No.:	
Expiry Date:	

DESCRIPTION

ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I is next generation master mix designed for one step real-time PCR reaction set up. The master mix is prepared in 2X concentrated solution and contains unique thermostable M-MULV enzyme, *Taq* DNA Polymerases, SYBR® Green dye, ROX dye as well as $MgCl_2$ and buffer components at optimal concentrations. The M-MULV enzyme has an optimal operating temperature and a higher affinity for primer template duplexes which allows very rapid processing during RT step. SYBR® Green dye emits fluorescence when bound to double-stranded DNA. Detection of PCR product is monitored by the increase in fluorescence, leading to high sensitivity, wide dynamic range and high reproducibility for quantification.

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ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I has several formulations optimized to be used with most of real-time PCR instruments. The sensitivity and consistency of ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I 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 SYBR® Green dye for intercalator-based qPCR
- Good buffer system for excellent amplification efficiency
- High sensitivity detection
- 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

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LIMITATION OF USE

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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	
QLMM14	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I (SYBR® Green Dye) 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™
QLMM14-LR	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with Low ROX (SYBR® Green Dye) Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7
QLMM14-R	ViPrimePLUS One Step *Taq* RT-qPCR Green Master Mix I with ROX (SYBR® Green Dye) 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)
Taq One Step RT-qPCR Green Master Mix I	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)
Taq One Step RT-qPCR Green Master Mix I	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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- 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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