ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix

Product code:	QLMM06
Packsize:	150 reactions

DESCRIPTION

ViPrimePLUS Lyophilised One Step qRT-PCR Master Mix is next generation first choice mix for a fast and easy one step real-time PCR reaction set up. The master mix has been freeze-dried to produce a room temperature stable preparation. The improved formulation of lyophilized master mix contains unique thermostable M-MULV (Moleney murine leukemia virus) enzyme, 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 qRT-PCR Master Mix is designed to achieve excellent results in reaction efficiency, correlation coefficient and slope.

ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix can be used to amplify any RNA template including mRNA, total RNA and viral sequences. The improved formulation of qRT-PCR can detect the extremely low copy number targets very specifically with high efficiency that give CT values close to the theoretical time of detection. The ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix is 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 Lyophilized One Step qRT-PCR Master Mix supplied with re-suspension buffer and ROX dye to be used with most of real-time PCR instruments which include hardware platforms that need ROX as a passive reference dye. The improved sensitivity and consistency of ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix in standard cycling conditions allow for industry leading performance in fast cycling conditions.

APPLICATIONS

All kinds of RNA sample material suited for qRT-PCR amplification can be used.

FEATURES

- Lyophilized form – room temperature stable preparation
- Fast and easy Real-Time PCR reaction set up
- Rapid extension rate for early Ct values
- Highest sensitivity and specificity
- Increased limit of detection
- Compatible on most of the real-time PCR platforms

COMPONENTS

3 x Lyophilized One Step qRT-PCR Master Mix
1 x Lyophilized ROX
4 x Re-suspension buffer

STORAGE

Store at ambient temperature on arrival and stable at ambient temperature up to the expiry date.
Once re-suspended with the provided buffer, the master mix should be stored at -20°C up to the expiry date stated. Keep in aliquot to reduce freeze-thaw cycles.

QUALITY CONTROL

As part of the ISO9001:2008 quality assurance systems, each lot of ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix 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.

PROTOCOL

Master Mix Preparation

1. Re-suspend each tube of Lyophilized One Step qRT-PCR Master Mix with 525µl of re-suspension buffers.
   a. Do not replace the re-suspension buffer with water or any other buffer.
   b. The master mix is ready to be used as a 2X qRT-PCR Master Mix.
2. ROX is required for platforms that use ROX as a passive reference guide. If ROX is required then follow the steps below.
   a. Re-suspend the tube of lyophilized ROX in the correct volume of re-suspension buffer according to the table below.
   b. Add the re-suspended ROX to each master mix at the correct level according to the table below.

Real-time PCR platforms	ROX re-suspension volume	ROX addition per tube of master mix
Applied Biosystems 7000, 7700, 7900, 7300 StepOne, StepOnePLUS and ViA7 platforms, Roche capillary Lightcyclers.	100µl	20µl
All Stratagene platforms	200µl	15µl
Applied Biosystems 7500 platform	700µl	10µl
All other machines	Not Required	Not Required

Reaction Preparation

1. Keep the qRT-PCR master mix protected from light until use it.
2. Aliquot the qRT-PCR 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 done and aliquot into tubes, place into qRT-PCR platform.

SUGGESTED MIXTURE

a. When using ViPrimePLUS gene detection kits:

Components	Reaction (1X)
ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix	10µl
Primer/Probe Mix	1µl
Template RNA	X µl
Nuclease free water	X µl
Final Volume	20µl

b. When using user supplied primers and probe:

Components	Reaction (1X)
ViPrimePLUS Lyophilized One Step qRT-PCR Master Mix	10µl
Primers (3pmols Forward & Reverse)	X µl
Probe (3pmols)	X µl
Template RNA (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	42°C	10mins
Enzyme activation	1	95°C	2mins
Denaturation	50	95°C	10secs
Data Collection*		60°C	60secs

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

b. For SYBR® green detection kits

Step	Cycles	Temp	Time
Reverse Transcription	1	42°C	10mins
Enzyme activation	1	95°C	2mins
Denaturation	50	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.

PREVENTION OF CONTAMINATION

qRT-PCR 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.

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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