Ampliqon

RealQ RT-PCR Master Mix One-Step kit (10mM $MgCl_2$)

(Final $MgCl_2$ is 5.0mM)

Cat. No.: 251510

Green DNA Dye already included in the Master Mix
ROX dye already included in the 2x RealQ Master Mix. No need to add ROX dye.

Cat. No.	Size Reactions	Kit
250403	200	RealQ-PCR Master Mix (3mM $MgCl_2$)
250406	200	RealQ-PCR Master Mix (6mM $MgCl_2$)
250407	200	RealQ-PCR Master Mix (7mM $MgCl_2$)
250410	200	RealQ-PCR Master Mix (10mM $MgCl_2$)
250503	200	RealQ-PCR Master Mix (3mM $MgCl_2$), *with Green DNA dye in mix*
250506	200	RealQ-PCR Master Mix (6mM $MgCl_2$), *with Green DNA dye in mix*
250507	200	RealQ-PCR Master Mix (7mM $MgCl_2$), *with Green DNA dye*
250510	200	RealQ-PCR Master Mix (10mM $MgCl_2$), *with Green DNA dye in mix*
250603	200	RealQ-PCR Master Mix (3mM $MgCl_2$), *with Green DNA dye in separate tube*
250606	200	RealQ-PCR Master Mix (6mM $MgCl_2$), *with Green DNA dye in separate tube*
250607	200	RealQ-PCR Master Mix (7mM $MgCl_2$), *with Green DNA dye in separate tube*
250610	200	RealQ-PCR Master Mix (10mM $MgCl_2$), *with Green DNA dye in separate tube*
250703	200	RealQ-PCR dUTP-UNG Master Mix (3mM $MgCl_2$)
250706	200	RealQ-PCR dUTP-UNG Master Mix (6mM $MgCl_2$)
250707	200	RealQ-PCR dUTP-UNG Master Mix (7mM $MgCl_2$)
250710	200	RealQ-PCR dUTP-UNG Master Mix (10mM $MgCl_2$)
250803	200	RealQ-PCR dUTP Master Mix (3mM $MgCl_2$)
250806	200	RealQ-PCR dUTP Master Mix (6mM $MgCl_2$)
250807	200	RealQ-PCR dUTP Master Mix (7mM $MgCl_2$)
250810	200	RealQ-PCR dUTP Master Mix (10mM $MgCl_2$)

Store at -20°C. *Reagent for in-vitro laboratory use only*

Important information for the User

Note: This protocol describes how to perform Real Time Reverse Transcription and PCR in a single step procedure. The Reverse Transcription and PCR reaction is performed in a single optimised buffer. This kit is intended for experience users that need high quality products to an affordable price. It is not the intention of this instruction insert to give a complete overview of the real time RT-PCR method, but simply a short guide describing the most important issues for running real time RT-PCR using RealQ RT-PCR products. For a more detailed description please consult the original manuals included with the Real Time PCR Instrument.

Components already included in the 2x RealQ RT-PCR One Step Master Mix: Optimized buffer system, ROX Reference dye, Green DNA dye, dATP, dCTP, dGTP and dTTP. The $MgCl_2$ concentration is 10mM, which gives an $MgCl_2$ concentration of 5mM in the final reaction.

Introduction

Ampliqon's RealQ RT-PCR One Step Kit can be used to perform absolute or relative quantisation of gene expression. The RealQ RT-PCR One Step kit is designed for the reverse transcription (RT) and amplification by PCR of a specific target RNA from either total RNA or mRNA.

Quantitative PCR and RT-PCR has become an important tool for SNP and gene expression analysis. Several different fluorescent chemistries exist for either detection of SNP's or quantitative gene transcripts. The use of fluorescent probe technologies reduces the risk of sample contamination while maintaining convenience, speed and high throughput screening capabilities.

One Step RT-PCR performs RT as well as PCR in a single buffer system. This offers the convenience of a single-tube preparation for RT and PCR amplification.

The RealQ Real RT-PCR Master Mix support quantitative amplification and detection with multiplex capability and show consistent high performance with various fluorescent detection systems including Green DNA I detection, TaqMan probes and Molecular beacons. The RealQ RT-PCR One Step kit has been designed for optimal performance on ABI PRISM™ Instruments, the LightCycler™ Instrument, the Mx4000™ Instrument and the DNA Engine Opticon™ System. The RealQ RT-PCR One Step kit includes the components necessary for performing cDNA synthesis of a specific target RNA and PCR amplification in a single step. The RealQ RT-PCR Enzyme mix includes Reverse Transcriptase and the TEMPase Hot Start DNA polymerase, a modified Taq DNA polymerase that is inactive during first strand synthesis and does not interfere. The TEMPase Hot Start enzyme is activated by a 15 minute at 95°C incubation step, which improves the PCR amplification reaction by decreasing background from non-specific amplification and increases amplification of desired products. The hot start also inactivates the reverse transcriptase enzyme, ensuring separation of reverse transcription and PCR.

Besides a universal 2X Master Mix RT-PCR One Step solution, several tubes are included containing RT-PCR Enzyme Mix, extra $MgCl_2$ and a special glass blocking agent for LightCycler™ Instrument users.

Materials provided for 200 Quantitative RT-PCR reactions (50 µl pr reaction)

Material provided (per kit)	Quantity
RealQ RT-PCR Enzyme Mix (50x)	200 µl
2x RealQ RT-PCR One Step Master Mix (10mM $MgCl_2$) *with Green DNA dye*	4 x 1.25 mL
Glass blocking agents (50X) (LightCycler™)	200 µl
$MgCl_2$ Concentration 25 mM	1.5 ml

Storage Conditions

Upon receipt, store all components at –20°C. Store the RT-PCR Enzyme Mix at –20°C. Store the 2X master mix at + 4°C after thawing. Once thawed, full activity is guaranteed for 3 month. Glass blocking agents and $MgCl_2$ can be stored at both -20°C and +4°C.

RNA Isolation

High-quality intact RNA is essential for successful synthesis of full-length cDNA. Isolation of polyA RNA is typically not necessary, although including this step may improve the yield of specific cDNA templates. RNA samples with an $OD_{260/280}$ of 1.8-2.0 are optimal.

PCR Pre-protocol considerations

RT-PCR One-Step Primers

Gene-specific primers anneal only to defined sequences and are use to synthesize cDNA from particular mRNA transcripts rather than from the entire mRNA population in the sample.
It is important especially in Green DNA dye I based real time RT-PCR applications to minimize the formation of non-specific amplification products. Especially at low target concentration it is important to use the lowest primer concentration without compromising the efficiency of RT and PCR. The optimal concentration of primer pairs is the lowest concentration that results in the lowest Ct and an adequate fluorescence for a given target concentration, with minimal or no formation of primer-dimer. The optimal concentrations of upstream and downstream primers are not always of equal molarity.

Primer concentration for TaqMan probes	Primer concentration for Molecular Beacons	Primer concentration in Multiplex PCR
50 to 600 nM	200 to 600 nM	20 to 200 nM

Primer concentration optimization scheme

RT-PCR One-Step probes

The optimal concentration of the experimental probe should be determined empirically. The optimal concentration is the lowest concentration that result in the lowest Ct and an adequate fluorescence for a given target concentration.

TaqMan probes, conc.	Molecular Beacons, conc.
Between 100 to 500 nM in increments of 100 nM	Between 200 to 500 nM in increments of 100 nM.

Probe concentration optimization scheme

Magnesium Chloride

The optimal $MgCl_2$ concentration gives maximal amplification of a specific target amplicon with minimal non-specific products and primer-dimer formation. It is important especially in Green DNA I dye based real time applications to optimize the $MgCl_2$ level, to avoid detection of non-specific dsDNA including primer-dimers. For TaqMan applications the optimization process is less important. In general the $MgCl_2$ concentration in Green DNA I dye based applications should be 5.0 mM. The master mix is supplied with a final $MgCl_2$ concentration of 5.0 mM. For adding extra $MgCl_2$ please consult the below table for one 50µl reaction.

Final $MgCl_2$ conc. in reaction (mM)	5.0	5.5	6.0	6.5	7.0
Additional volume of 25 mM $MgCl_2$ per 50µl reaction (µL):	0	1	2	3	4

$MgCl_2$ dilution scheme

Reference Dye

A passive reference dye is included in the 2x RealQ Master Mix kit to compensate for non-PCR related variations in the fluorescence. The fluorescence from the passive reference dye does not change during the course of the PCR reaction but provide a stable baseline to which samples are normalized. The excitation and emission of the reference dye are 584 nm and 612 nm, respectively.

Preventing Template Cross-Contamination

Due to the high sensitivity of real time RT-PCR it is a risk that reaction may be contaminated with the products of previous runs. To minimize this risk, tubes or plates containing reaction products should not be opened or analyzed by gel electrophoresis in the same laboratory area used to set up reactions.

Glass blocking agents (LightCycler™)

One extra challenge using the LightCycler™ instrument is the precipitation of RT-PCR reagent at the glass capillary surface as the real time RT-PCR progresses. To prevent this event Ampliqon has design a special reagent for blocking the glass capillaries during the real time RT-PCR reaction. The Glass blocking agent comes as a 50X solution (1 µl pr 50 µl PCR reaction).

Protocol for Real-Time RT-PCR

Prior to the experiment, it is prudent to carefully optimize experiment conditions and to include controls at every stage. See pre-protocol considerations for details.

If the Green DNA I dye will be included in the reaction consult pre-protocols consideration for details.

Thaw the RealQ RT-PCR Master Mix and store on ice. Following initial thawing of the master mix, store the unused portion at +4°C.

Note: Multiple freeze-thaw cycles should be avoided. The Green DNA I dye if present in the master mix is light sensitive. Solution containing the Green DNA I dye should be protected from light whenever possible.

Prepare the experimental reaction by adding the following components in order:

25 µl of 2X RealQ RT-PCR Master Mix
x µl of experimental probe (optimized concentration)
x µl of upstream primer (optimized concentration)
x µl of downstream primer (optimized concentration)
1 µl of RealQ RT-PCR Enzyme Mix (50X)

Gently mix the reactions without creating bubbles (do not vortex).
Add x µl of Total RNA or mRNA to each experimental reaction.

Add Nuclease-free PCR-grade H_2O to adjust the final volume to 50µl (including experimental DNA)

Gently mix the reaction without creating bubbles (do not vortex).

Note: Bubbles interfere with fluorescence detection.

Place the reaction in the instrument and run the appropriate program below.

2-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	30 minutes	55 °C
1[b]	15 minutes	95 °C
40	15-30 seconds[c]	95 °C
	1.0 minutes[d]	55-60 °C[e]

[a] For synthesis of the gene-specific cDNA from Total RNA or mRNA

[b] For activation of the TEMPase Hot Start enzyme.

[c] Varying between thermocycles, use 30 seconds for the ABI PRISM 7700 instrument.

[d] Set the temperature cycler to detect and report fluorescence during the annealing/extension step of each cycle.

[e] Choose an appropriate annealing temperature for the primer set used.

3-step PCR Program

Cycles	Duration of cycle	Temperature
1[a]	30 minutes	55 °C
1[b]	15 minutes	95 °C
40	30 seconds	95 °C
	1.0 minute[d]	55-60 °C
	30 seconds	72 °C

Related Products

Description	Cat. No.
Taq DNA Polymerase (500 Units) *with 10X Ammonium Reaction Buffer* *with 10X Standard Reaction Buffer*	**110303**
Taq DNA Polymerase (500 Units) *with 10X Combination Buffer*	**110403**
Taq DNA Polymerase (500 Units) *with 10X Mg^{++} Free Ammonium Buffer*	**110503**
Taq DNA Polymerase 2.0X Master Mix (100 Reac) *with 2.0 mM MgCl2*	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 Reac) *with 1.5 mM MgCl2,*	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) *with 2.0 mM MgCl2*	**190301**
AccuPOL DNA Polymerase (500 Units)	**210303**
TEMPase Hot Start DNA Polymerase (500Units) *with 10X TEMPase Buffer I* *with 10X TEMPase Buffer II*	**220303**
UniPOL –Long Range PCR (100 Reac)	**270701**
Rapid Ligation Kit (50 React)	**750300**
RT-PCR One Tube (100 Reac)	**740301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer I* (100 Reac)	**230301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer II* (100 Reac)	**230701**
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	**501004**
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	**502004**
GC5 Value Efficiency, 10^8 Cfu/µg pUC19 Chemically Competent Cells, (10x 200µl)	**812010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (10x 50µl)	**805010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (5x 200µl)	**802005**
SuperPath GC10, 10^{10} Cfu/µg pUC19 ElectroCompetent Cells, (5x 80µl)	**830805**
SOC Medium, 10x 10mL	**800000**

NOTICE

In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.