



## RealQ-PCR Master Mix Kit (7mM MgCl<sub>2</sub>)

(Final MgCl<sub>2</sub> is 3.5mM)

For 200 Reactions of 50µl pr Reactions

Cat. No.: 250607

**Green DNA Dye in separate tube.**

**ROX dye already included in the 2x AmpliQ Master Mix. No need to add ROX dye.**

Cat. No.	Size Reactions	Kit
250403	200	RealQ-PCR Master Mix (3mM MgCl <sub>2</sub> )
250406	200	RealQ-PCR Master Mix (6mM MgCl <sub>2</sub> )
250407	200	RealQ-PCR Master Mix (7mM MgCl <sub>2</sub> )
250410	200	RealQ-PCR Master Mix (10mM MgCl <sub>2</sub> )
250503	200	RealQ-PCR Master Mix (3mM MgCl <sub>2</sub> ), with Green DNA dye in mix
250506	200	RealQ-PCR Master Mix (6mM MgCl <sub>2</sub> ), with Green DNA dye in mix
250507	200	RealQ-PCR Master Mix (7mM MgCl <sub>2</sub> ), with Green DNA dye
250510	200	RealQ-PCR Master Mix (10mM MgCl <sub>2</sub> ), with Green DNA dye in mix
250603	200	RealQ-PCR Master Mix (3mM MgCl <sub>2</sub> ), with Green DNA dye in separate tube
250606	200	RealQ-PCR Master Mix (6mM MgCl <sub>2</sub> ), with Green DNA dye in separate tube
250607	200	RealQ-PCR Master Mix (7mM MgCl <sub>2</sub> ), with Green DNA dye in separate tube
250610	200	RealQ-PCR Master Mix (10mM MgCl <sub>2</sub> ), with Green DNA dye in separate tube

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

### Important information for the user

This kit is intended for more experience users that needs high quality products to an affordable price. It is not the intention of this instruction insert to give a complete overview of the Quantitative PCR methods but simply a short guide describing the most important issues for running Quantitative PCR using RealQ-PCR products. For more detailed description please consult the original manuals coming with the Quantitative PCR Instrument. Components already included in the 2x RealQ-PCR Master Mix: Optimized buffer system, ROX Reference dye, dATP, dCTP, dGTP and dTTP. The MgCl<sub>2</sub> concentration is 7mM, which gives an MgCl<sub>2</sub> concentration of 3.5mM in the final reaction.

### Introduction

Quantitative PCR has become an important tool for SNP and gene expression analysis. Several different fluorescent chemistries exist for either detection of SNP 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. Ampliqon has developed the RealQ-PCR Master Mix, a single-tube 2X reagent ideal for most Quantitative PCR applications. The RealQ-PCR kit support quantitative amplification and detection with multiplex capability. The RealQ-PCR 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-PCR kits includes the components necessary for performing PCR amplification, and have been successfully used to amplify and detect a variety of DNA targets such as genomic DNA, cDNA and plasmid DNA.

The RealQ-PCR master mix includes the TEMPase Hot Start DNA polymerase, a modified Taq DNA polymerase with hot start capabilities. The TEMPase Hot Start enzyme improves the PCR amplification reaction by decreasing background from non-specific amplification and increases amplification of desired products.

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

Materials provided (per kit)	Quantity
2X RealQ PCR Master Mix (7mM MgCl <sub>2</sub> )	4 x 1.25 mL
Green DNA Dye I (10.000x)	10 µL
50X Glass blocking agents (LightCycler™)	200 µL
MgCl <sub>2</sub> Concentration: 25 mM	1.5 mL

### Storage Conditions

Upon receipt, store all components at -20°C. Store the 2X master mix at + 4°C after thawing. Once thawed, full activity is guaranteed for 3 month. The Green DNA dye I should be stored at -20°C. The Green DNA dye I is not stable in diluted form more than 1-3 day at +4 °C. Glass blocking agents and MgCl<sub>2</sub> can be stored at both -20°C and +4°C. The Green DNA dye I are light sensitive and should be kept away from light whenever possible.

## PRE-PROTOCOL CONSIDERATIONS:

### PCR Primers

It is important especially in Green DNA dye I based Quantitative 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 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	Primer concentration for use in Multiplex PCR
50 to 600 nM	20 to 200 nM

*Primer 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 Quantitative applications to optimized the  $MgCl_2$  level, to avoid detection of non-specific dsDNA including primer-dimers. In general the  $MgCl_2$  concentration in Green DNA I dye based application should be between 1.5 and 5.0 mM. The master mix is supplied with a final  $MgCl_2$  concentration of 3.5 mM. For adding extra  $MgCl_2$  please consult the below table.

Final $MgCl_2$ conc. in reaction (mM)	3.5	4.0	4.5	5.0
Additional volume of 25 mM $MgCl_2$ per 50 $\mu$ l reaction ( $\mu$ L):	0	1	2	3

*$MgCl_2$  dilution scheme*

### Green DNA dye I Dilution Recommendations

Prepare fresh dilutions of the Green DNA dye I prior to setting up the reactions, and keep all tubes containing the Green dye protected from light. Make initial dilutions of the Green DNA dye using nuclease-free PCR-grade  $H_2O$ . If you are amplifying a short amplicon (50-400 bp) use the Green DNA dye in a final dilution of 0.5X. If you are amplifying a long amplicon (400-900 bp), use the Green DNA dye in a final dilution of (1/6)X in the reaction. The Green DNA dye is supplied at 10.000X.

Green DNA dye I in a 50 $\mu$ l PCR reaction:
Short target (between 50-400 bp) pre-dilute 1:2000 add 5.0 $\mu$ l
Long target (between 400-900 bp) pre-dilute 1:3000 add 2.5 $\mu$ l.

*Green DNA dye dilution scheme*

### Reference Dye

A passive reference dye is included in the 2x RealQ-PCR 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 Quantitative 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 that the PCR reagents can form precipitate on the glass capillary surface as the Real time PCR progresses. To prevent this event Ampliqon has designed a special reagent for blocking the glass capillaries during the Quantitative PCR reaction. The Glass blocking agent comes as a 50X solution (1  $\mu$ l pr 50  $\mu$ l PCR reaction).

### Protocol

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.

Thaw the 2x RealQ-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 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 components in the following order:

- 25  $\mu$ l of 2X master mix
- x  $\mu$ l of experimental probe (optimized concentration)
- x  $\mu$ l of upstream primer (optimized concentration)
- x  $\mu$ l of downstream primer (optimized concentration)

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

Add x  $\mu$ l of experimental gDNA, cDNA or plasmid DNA to each experimental reaction.

Add Nuclease-free PCR-grade  $H_2O$  to adjust the final volume to 50 $\mu$ l (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 <sup>a</sup>	2 minutes	50 °C
1 <sup>b</sup>	15 minutes	95 °C
40	15-30 seconds <sup>c</sup>	95 °C
	1.0 minute <sup>d</sup>	55-60 °C <sup>e</sup>

<sup>a</sup> Can be excluded if UNG is not used.

<sup>b</sup> For activation of the TEMPase I hot start enzyme.

<sup>c</sup> Varying between thermocycles, used 30 seconds for the ABI PRISM 7700 instrument.

<sup>d</sup> Set the temperature cycler to detect and report fluorescence during the annealing/extension step of each cycle.

<sup>e</sup> Choose an appropriate annealing temperature for the primer set used.

## 3-step PCR Program

Cycles	Duration of cycle	Temperature
1 <sup>a</sup>	2 minutes	50 °C
1 <sup>b</sup>	15 minutes	95 °C
40	30 seconds	95 °C
	1.0 minute <sup>d</sup>	55-60 °C <sup>e</sup>
	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 <sup>++</sup> Free Ammonium Buffer	110503
Taq DNA Polymerase 2.0X Master Mix (100 Reac) with 2.0 mM MgCl <sub>2</sub>	150301
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) with 1.5 mM MgCl <sub>2</sub>	180301
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) with 2.0 mM MgCl <sub>2</sub>	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
Real-Time PCR Master Mix (200 Reac)	260602
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
AmpliQ Real Time PCR Master Mix , (200 Reac) Green DNA dye I included, 10mM MgCl <sub>2</sub> ,	260602
AmpliQ Real Time PCR Master Mix , (200 Reac) Green DNA dye I included, 3mM MgCl <sub>2</sub> ,	260605
AmpliQ Real Time PCR Master Mix , (200 Raec) Green DNA dye I included, 6mM MgCl <sub>2</sub> ,	260608
AmpliQ Real Time PCR Master Mix (200 Reac) 10mM MgCl <sub>2</sub> , ex Dye	260612
AmpliQ Real Time PCR Master Mix ,(200 Reac) 3mM MgCl <sub>2</sub> , ex Dye	260615
AmpliQ Real Time PCR Master Mix , (200 Reac) 6mM MgCl <sub>2</sub> , ex Dye	260618
AmpliQ Real Time PCR Master Mix, (200 Reac) 10mM MgCl <sub>2</sub> , Green DNA Dye	260622
AmpliQ Real Time Single Step RT-PCR MaMi 10mM MgCl <sub>2</sub> , Green DNA dye I, (200 Reac)	260902
AmpliQ Real Time Single Step RT-PCR MaMi, Green DNA dye I, 3mM MgCl <sub>2</sub> (200 Reac)	260905
AmpliQ Real Time Single Step RT-PCR MaMi, Green DNA dye I, 6mM MgCl <sub>2</sub> (200 Reac)	260908
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 <sup>8</sup> CfU/µg pUC19 Chemically Competent Cells, (10x 200µl)	812010
GC5 High Efficiency, 10 <sup>9</sup> CfU/µg pUC19 Chemically Competent Cells, (10x 50µl)	805010
GC5 High Efficiency, 10 <sup>9</sup> CfU/µg pUC19 Chemically Competent Cells, (5x 200µl)	802005
SuperPath GC10, 10 <sup>10</sup> CfU/µg pUC19 ElectroCompetent Cells, (5x 80µl)	830805
SOC Medium, 10x 10mL	800000

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TaqMan® is a registered trademark of Roche Molecular Systems, Inc.

Tween® 20 is a registered trademark of ICI Americas, Inc.

Mx4000 is a registered trademark of Stratagene Corporation

### 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.