

## Universal Real Time PCR

### Product Description

Universal Real Time PCR Master Mix Kit  
For 200 Reactions of 50µl pr Reactions

Catalog Number	Kit	Size Reactions
UPAP160A	Real-Time PCR Master Mix	200
UPAP160B	Real-Time ROX Reference	200

**Storage:** at -20°C FOR RESEARCH USE ONLY

### Scientific and Technical Information

Quantitative PCR has becoming 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. The Real Time PCR Master Mix, a single-tube 2X reagent has been developed to be ideal for most real time PCR applications. The Real Time PCR kit 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 Real Time 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 Real Time PCR kit includes the components necessary for performing PCR amplification, and have been successfully used for amplify and detect a variety of DNA target such as genomic DNA, cDNA and plasmid DNA.

The Real Time PCR master mix includes the TEMPase I DNA polymerase, a modified Taq DNA polymerase with hot start capabilities. The TEMPase I enzyme improves the PCR amplification reaction by decreasing background from non-specific amplification and increases amplification of desired products. To prevent template cross-contamination the master mix are design for future UNG (Uracil-N-glycosylase) treatment by replacement of dTTP with dUTP, however the master mix does not contain the UNG enzyme in present kit version.

To create a universal Real Time PCR kit, besides the 2X master mix solution, several separate tubes are included containing a passive Reference dye (ROX), the Green DNA I dye, extra MgCl<sub>2</sub> and final a special glass blocking agents for LightCycler™ Instrument users.

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 real time PCR methods but simply a short guide describing the most important issues for running real time PCR using Real Time PCR products. For more detailed description please consult the original manuals coming with the Real Time PCR Instrument.

#### **Materials provided for 200 Real Time PCR reactions (50 µl pr reaction)**

Materials provided (per kit)	Quantity
2x Real Time PCR Master Mix	4 x 1.25 ml
Reference dye	200 µl
10.000x Green DNA dye I	10 µl
50x Glass blocking agents (LightCycler™)	200 µl
MgCl <sub>2</sub> Concentration: 25 mM	1.5 ml

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**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. It is possible to include the Reference dye in all 4 x 1.25 ml master mix at once and store at +4°C with same stability.  
 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 Reference dye and the Green DNA dye I are light sensitive and should be kept away from light whenever possible.

**Directions for Use**

**Pre-Protocol Considerations :**

**PCR Primers**

It is important especially in Green DNA dye I based real time 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 for use with TaqMan probes	Primer concentration for use with Molecular Beacons	Primer concentration for use in Multiplex PCR
50 to 600 nM	200 to 600 nM	20 to 200 nM

Primer concentration optimization scheme.

**PCR 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 prober, 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<sub>2</sub> 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 optimized the MgCl<sub>2</sub> level, to avoid detection of non-specific dsDNA including primer-dimers. For TaqMan application the optimization process is less important. In general the MgCl<sub>2</sub> concentration in Green DNA I dye based application should be between 1.5 and 2.5 mM while for TaqMan application should be between 2.5- 5.0 mM. The master mix is supplied with a final MgCl<sub>2</sub> concentration of 1.5 mM. For adding extra MgCl<sub>2</sub> please consult the below table.

Final MgCl <sub>2</sub> conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl <sub>2</sub> per reaction (µL):	0	1	2	3	4	5	6

MgCl<sub>2</sub> 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<sub>2</sub>O. 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 µl PCR reaction:
Short target (between 50-400 bp) pre-dilute 1:2000 add 5.0 µl
Long target (between 400-900 bp) pre-dilute 1:3000 add 2.5 µl.

Green DNA dye dilution scheme

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### Reference Dye

A passive reference dye is included in this kit and may be added 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.

ABI PRISM 7700 or GeneAmp 5700 users	Mx4000 users
Add 1 µl to a 50 µl PCR reaction, final dilution 50X	Pre-dilute 1:10 and add 1 µl to a 50 µl PCR reaction, final dilution 500X

Reference dye dilution scheme

### Preventing Template Cross-Contamination

Due to the high sensitivity of real time 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. dUTP is included in the realtime PCR master mix to prevent PCR products from becoming source of contamination. Reaction samples can be treated with the heat labile uracil-N-glycosylase (UNG) prior to PCR. The UNG enzyme are not included in the master mix, but can be added in a concentration of 1units pr 50 µl PCR reaction.

### Glass blocking agents (LightCycler™)

One extra challenge using the LightCycler™ instrument is the precipitate of PCR reagent at the glass capillary surface as the real time PCR progressed. To prevent this event a special reagent for blocking the glass capillaries during the real time PCR reaction has been designed. The Glass blocking agent comes as a 50X solution (1 µl pr 50 µl 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.

If the reference dye will be included in the reaction (optional), dilute 1:50 (ABI PRISM 7700/GeneAmp 5700).

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

To obtained same performance as the TaqMan Universal PCR Master Mix from Applied Biosystem, adjust the MgCl<sub>2</sub> concentration to 5mM by adding 7.0µl pr 50µl PCR reaction.

Thaw the Real Time 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 master mix
- x µl of experimental probe (optimized concentration)
- x µl of upstream primer (optimized concentration)
- x µl of downstream primer (optimized concentration)
- 1µl of Reference dye

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

Add x µl of experimental gDNA, cDNA or plasmid DNA to each experimental reaction.

Add Nuclease-free PCR-grade H<sub>2</sub>O 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.

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**FT-UPAP160A**
**2-step PCR Program**

Cycles	Duration of cycle	Temperature
1 <sup>a</sup>	2 minutes	50 °C
1 <sup>b</sup>	10 minutes	95 °C
40	15-30 seconds <sup>c</sup>	95 °C
	1.0 minutes <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 cyclers 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>	10 minutes	95 °C
40	30 seconds	95 °C
	1.0 minutes <sup>d</sup>	55-60 °C <sup>e</sup>
	30 seconds	72 °C

## Other Information

**Related Products**

Product	Catalog Number	Quantity
UptiTherm DNA Polymerase 5U/μl with Mg free Buffer + 50 mM MgCl <sub>2</sub> buffer	UPS53921	1000 Units
UptiTherm DNA Polymerase 5 U/μl with 2 mM MgCl <sub>2</sub> Buffer	UPS53881	1000 Units
UptiTherm DNA Polymerase gel form with Mg free Buffer + dNTP in 0,2 ml PCR tube	UPS54081	50 Units
UptiTherm DNA Polymerase gel form with 2 mM MgCl <sub>2</sub> Buffer + dNTP in 0,2 ml PCR tube	UPS54071	50 Units
UptiPfu DNA Polymerase 1U/μl with Mg free Buffer + 50 mM MgCl <sub>2</sub> buffer	UPAK5105	500 Units
UptiPfu DNA Polymerase 1U/μl with 2 mM MgCl <sub>2</sub> Buffer	UPAK5102	500 Units
UptiTherm Hot Start PCR Master Mix	UPQ6587A	250 Units
RedTAQ DNA Polymerase	UPAP1221	1000 Units
Rapid Ligation Kit	UPN14171	50 Reactions
RT-PCR One Tube	UPS53944	100 Reactions
UptiTherm Complete Mg buffer	UPS54071	50 reactions
UptiTherm Complete Mg free	UPS54081	50 reactions
RedTaq DNA pol Mg free	UPAP1221	1000 reactions
PCR Optimizer Kit	UPAP2400	3 x 1.5 ml
Enhancer PCR Optimizer Kit	UPAP2410	10 reactions
PCR Mix 3 (10 mM of each dA, dC, dG and dT)	UP984440	200μl
GC5 Value Efficiency, 10 <sup>8</sup> CfU/μg pUC19 Chemically Competent Cells	UPAM893A	10x 200μl
GC5 High Efficiency, 10 <sup>9</sup> CfU/μg pUC19 Chemically Competent Cells	UPAM889B	10x 50μl
GC5 High Efficiency, 10 <sup>9</sup> CfU/μg pUC19 Chemically Competent Cells	UPAM889A	20x 50μl
GC5 High Efficiency, 10 <sup>9</sup> CfU/μg pUC19 Chemically Competent Cells	UPAM889C	5x 200μl

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SuperPath GC10, 10 <sup>10</sup> Cfu/μg pUC19 ElectroCompetent Cells	UPAM885A	5x 80μl
SuperPath GC10, 10 <sup>10</sup> Cfu/μg pUC19 ElectroCompetent Cells	UPAM885B	75x 80μl
SuperPath GC10, 10 <sup>10</sup> Cfu/μg pUC19 ElectroCompetent Cells	UPAM885C	5x 100μl
SuperPath GC10, 10 <sup>10</sup> Cfu/μg pUC19 ElectroCompetent Cells	UPAM885D	75x 100μl
SOC Medium	UPAN146A	10x 10ml

ABI PRISM® is a registered trademark of PE Corporation.  
 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.

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