



RealQ Plus 2x Master Mix Green Dye, High ROX

Cat. No.	Reactions	RealQ Plus 2x Master Mix
AO325402	400	4 x 1.25 ml
AO325406	4,000	40 x 1.25 ml

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

Detection Limit

Approximately 1 copy

Quantification Limit

Approximately 24 copies (0.08 ng of human gDNA correlating to 12 diploid genomes with 2 gene copies per diploid genome)

Compatibility

Real-time instruments which require high ROX as internal reference dye e.g. StepOne and StepOne Plus from Life Technologies.

General Description

Quantitative PCR is an important tool for SNP and gene expression analysis. Two general fluorescent chemistries exist for quantitative detection of gene transcripts: probes (e.g. TaqMan®, Scorpions™ Probes, molecular beacons) and DNA-binding fluorescent dyes (e.g. ethidium bromide, SYBR® Green, EvaGreen®, PicoGreen®). AS ONE offers the RealQ Plus 2x Master Mix in two formulations: for probe and including DNA-binding fluorescent dye, making them ideal for most quantitative PCR applications.

The RealQ Plus 2x Master Mixes are available with high, low or without ROX for optimal performance on most of the commonly used real-time PCR instruments. The RealQ Plus 2x Master Mix promotes high specificity and low background by using Hot Start DNA Polymerase, a modified Taq DNA polymerase with hot start capabilities.

The RealQ Plus 2x Master Mix Green Dye with High ROX is a single-tube 2x reagent including all components necessary to perform real-time DNA amplification for DNA-binding dye based PCR. Just add your primers and DNA. The ROX internal reference dye level is optimized for real-time instruments that require high ROX as internal reference dye e.g. StepOne and StepOne Plus from Thermo Fisher Scientific.

Composition of RealQ Plus 2x Mix Green Dye with High ROX

- HS DNA Polymerase
- Optimized buffer system including dNTPs, green dye, and ROX reference dye

Storage and Stability

Unopened product is stable at -20°C for 2 years. Store the master mix at 4°C after thawing. Once thawed, full activity is guaranteed for 3 months.

Quality Control

HS DNA Polymerase is tested for contaminating activities, with no trace of endonuclease activity, nicking activity, exonuclease activity or priming activity. The RealQ Plus 2x Master Mix Green Dye with High ROX is functionally tested for efficiency and absence of contaminating human genomic DNA.

Pre-Protocol Considerations

- ROX™: ROX is used as passive reference dye to compensate for non-PCR related variations in the fluorescence. The ROX fluorescence does not change during the course of the PCR reaction nor does it influence the PCR reaction. It provides a stable baseline to which samples are normalized. The RealQ Plus 2x Master Mix Green Dye with High ROX is optimized to be used with real-time instruments which require high ROX as internal reference dye e.g. StepOne and StepOne Plus.
- PCR Primers: It is important - especially in fluorescent DNA dye based quantitative PCR applications - to minimize the formation of non-specific amplification products. Particularly at low target concentration it is important to use the lowest possible primer concentration without compromising the efficiency of the PCR. The optimal concentration of primer pairs is the lowest concentration that results in the lowest C_t and adequate fluorescence for a given target concentration with minimal or no formation of primer-dimers. The optimal concentrations of upstream and downstream primers are not always of equal molarity. Optimal concentrations of primers are in the range of 50 nM to 600 nM.
- Cross-Contamination: Due to the high sensitivity of quantitative PCR, there is a risk of contaminating the reactions 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.

Suggested Protocol for RealQ Plus 2x Mix Green Dye with high ROX

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

- Prior to the experiment, it is crucial to carefully optimize experimental conditions and to include the controls at every stage.
- Thaw the RealQ Plus 2x Master Mix Green Dye with High ROX. Following initial thawing of master mix, store the

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unused portion at 4°C. Multiple freeze-thaw cycles should be avoided.

1. Prepare the experimental reaction by adding the components in the order shown in the table below.

Component	Vol./reaction	Final Conc.
RealQ Plus 2x Master Mix	12.5 µL	1X
Primer A (10 µM)	0.5 µL (0.25-2.5 µL)	0.1 µM (0.05–0.5µM)
Primer B (10 µM)	0.5 µL (0.25-2.5 µL)	0.1 µM (0.05–0.5µM)
PCR-grade Water	Variable	----
Template DNA	Variable	genomic DNA: 20 ng (1 – 100 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	25 µL	----

2. Mix gently by pipetting the solution up and down a few times without creating bubbles. (Do not vortex).
3. Program the thermal cycler according to the manufacturer's instructions. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
4. Place the tubes in the thermal cycler and start the reaction.

Three-step PCR program

Cycles	Duration of cycle	Temperature
1 ^a	15 minutes	95°C
40	15-30 seconds ^b 30 seconds ^c 30 seconds	95°C 55-60°C ^d 72°C

Two-step PCR program (recommended)

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

- ^a For activation of the hot start enzyme.
^b Denaturation time may vary between thermocyclers.
^c Set the qPCR instrument to detect and report fluorescence during the annealing/extension step of each cycle.
^d Choose an appropriate annealing temperature for the primer set used.

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