# RealQ Plus 2x Master Mix

## Green Dye, *without ROX*

| Cat. No. | Reactions | RealQ Plus 2x Master Mix |
|---|---|---|
| **AO323402** | 400 | 4 x 1.25 ml |
| **AO323406** | 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 with no requirements for normalization to an internal reference dye.

### *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 without ROX is a single-tube 2x reagent including all components necessary to perform DNA-binding dye based real-time DNA amplification. You just need to add your primers and DNA.

### *Composition of RealQ Plus 2x Mix Green Dye without ROX*

- HS DNA Polymerase
- Optimized buffer system including dNTPs and Green 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 without ROX is functionally tested for efficiency and absence of contaminating human genomic DNA.

### *Pre-Protocol Considerations*

- 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 without 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 without ROX. Following initial thawing of master mix, store the 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)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>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]<br>30 seconds[c]<br>30 seconds | 95°C<br>55-60°C[d]<br>72°C |

**Two-step PCR program (recommended)**

| Cycles | Duration of cycle | Temperature |
|---|---|---|
| 1[a] | 15 minutes | 95°C |
| 40 | 15-30 seconds[b]<br>60 seconds[c] | 95°C<br>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.