# EQ 2x qPCR Master Mix Green kit, w/o ROX™

## Cat No: RN014S

EQ 2x qPCR Master Mix Green is a reliable and robust real time PCR mix that is composed of a Hot Start DNA Polymerase, dNTPs, green-fluorescent dye and an optimized buffer system. It has many applications in molecular analyses of DNA and RNA (cDNA) substrates, and it has been tested in commonly used real-time PCR instruments.

Tested for bacterial, human genomic DNA and human cDNA from HeLa cells, over at least 5 logs, of starting quantities of 1 ng (~ 2 X 10^5 copies), 64ng (~ 20 000 copies) and 40 ng respectively, the kit provides a high quantification efficiency (Efficiency >95%, and $R^2$ >0.99) down to 1 copy of genomic DNA (4-6 copies of target genes) and 0.25 pg of cDNA templates (from Total RNA).

One of EQ 2x qPCR Master Mix Green's most important features is its stability at 2-8 $^0$C for at least 3 months after thawing, during which there is no observable loss of amplification efficiency.

**Form:** Liquid

### Characteristics

- Easy to use for qualitative and quantitative assays. Just add Primers and Template!
- Very low fluorescence background
- Highly specific, stable, reproducible and efficient
- Reaction set-up at room temperature
- Pre-mixed all-in-one 2x solution

Version 3.0/ Jan-26

## Product Details

### 500 qPCR Reactions of 20 µl

**Reagents supplied:**

| Cat no: RN014S | | |
|---|---|---|
| **Cat No** | **Description** | **Tubes No.** |
| **RN013-1.3** | **EQ 2x qPCR Master Mix Green, w/o ROX, 1.3 ml** | 4 |
| **BR033-1.8** | **Nuclease Free Water, 1.8 ml** | 2 |



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**Quality Control:** The EQ 2x qPCR Master Mix Green, w/o ROX™ is functionally tested for its efficiency, as well as its absence of contaminating bacterial and human genomic DNA.

**Storage**: For long-term storage, store at -20 °C. After thawing, store at 2-8°C for up to 3 months. Avoid multiple freeze-thaw cycles. Do not freeze-thaw more than 2 times.

**Instrument compatibility:**
Real-time instruments that do not require ROX™ internal reference dye as for example: Bio-Rad CFX OPUS Touch™, CFX96 Touch™, CFX384 Touch™, CFX Connect™, DNA Engine Opticon® 2, Chromo4™, iCycler iQ™ and My iQ™, Roche LightCycler® 480, LightCycler® 1536, LightCycler® Nano, LightCycler® 96 and QuantStudio™ instruments, Thermo Scientific™ PikoReal™, Cepheid SmartCycler®, Bio Molecular Systems Mic qPCR cycler, Qiagen Rotor Gene Q, Rotor Gene 6000, MyGo Mini and MyGo Pro

**Important Notes:**

- Thaw the EQ 2x qPCR Master Mix Green on ice or at 2-8 °C. Following the initial thawing, store the remaining quantity at 4 °C.
- Avoid Freeze-thaw for optimal results! Quantification efficiency may drop by 5-8 % after 2 freeze-thaw cycles.
- Solutions containing fluorescent green intercalating dyes (EQ 2x qPCR Master Mix, w/o ROX) should be protected from light whenever possible.



## General tips for the Reactions' setup

**PCR Primers**
It is of high importance, especially in fluorescent dye-based qPCR applications, to minimize the formation of non-specific amplification products during early cycles. At low target concentration, it is preferable to use lower primer concentration in order to minimize the primer to template ratio and potentially avoid unwanted product accumulation. As a general rule, the optimal primer concentration is the lowest concentration that produces the lowest Cq value and adequate fluorescence accumulation, while having a minimal or no formation of primer-dimers. The optimal concentration of primers may be in the range of 100-800 nM.


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**Annealing temperature**

Annealing temperature should be adjusted for each primer-set by a gradient program which takes under consideration the Tm estimated by a Tm calculator (like oligoanalyzer on the IDT website) that calculates the Tm value based on the salt concentration, the $MgCl_2$ and the dNTPs concentration. Ideally, both primers should have a similar Tm value ~ 60-65 °C.

Set a 2 or 3-step qPCR program (depending on your application) with a gradient at annealing/extension phase that includes the following temperatures:

a) Tm °C
b) Tm -2 °C
c) Tm -4 °C
d) Tm-8 °C

where "Tm" is the melting temperature produced by the software (example: oligoanalyzer by IDT). If primers have a Tm temperature difference**, pick the lowest Tm value.**

In each temperature, set reactions with a certain template concentration and a 1:10 dilution in triplicates each plus a negative control in duplicates with no template (8 reactions per temperature, 32 reactions in total). The program should include a melting curve mode after the cycling phase to record any non-specific products.

Select as annealing temperature the temperature that:

a) produces the optimal curve with the highest plateau,
b) the minimum Cq values,
c) the closest to the expected difference between average Cq values that were obtained for 1 and 1:10 template dilution (Closest to 3.248-3.394) and
d) produces no primer dimers and/or unspecific products, that may be detected during the melting curve stage.

**Avoiding Contamination**

**One should never open the PCR tubes, after the end of the qPCR program, in the same are**a (laboratory bench or preferably the same room**) where the master mix is prepared to** avoid DNA contamination from amplification products. In cases, where gel analysis is required, it is highly suggested to:

a) allow the tube to cool down to 10-25 °C (room temperature) and spin down for 20 seconds at max speed before opening the caps
b) Prepare and analyze the gels at a different area from the real-time PCR setup area



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c) Use a different pipette, solely for gel loading and never use the same pipette for end point or real-time setup
d) Disinfect the bench and pipettes using a paper towel and a light spritz of a 10% bleach solution. Then, rinse well with a 70% Ethanol solution.

## Protocol

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

| Component (Stock concentration) | Volume/Standard Reaction (proposed range) | Final Concentration in Standard Reaction (proposed range) |
|---|---|---|
| **Nuclease Free Water** | X µl | N/A |
| **Primer A (10 µM)** | 0.8 µl (0.2 -1.6 µl) | 0.4 µM (0.1-0.8 µM) * |
| **Primer B (10 µM)** | 0.8 µl (0.2 -1.6 µl) | 0.4 µM (0.1-0.8 µM) * |
| **EQ 2x qPCR Master Mix Green, w/o ROX** | 10 µl (you can also set 10 µl reaction by adding 5 µL and adjusting all other ingredients | 1 X |
| **Template DNA**** | Y µl (when high levels of PCR inhibitors are expected, do not use more than 10% of the reaction Volume) | Human Genomic DNA :10 ng (100 ng-6 pg)**<br>*E. coli* Genomic DNA: 1 ng (10ng - 10 fg)**<br>cDNA: 50 ng (100 ng-0.2 pg)**<br>Plasmid DNA : 0.5 ng (down to 3-5 copies) |
| **Total Reaction Volume***** | 20 µl (or 10 µl) | N/A |

***Table 1.*** *qPCR Reactions setup.*

***Optimization is highly recommended (see above section "annealing temperature")***



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***** The indicated quantities have been tested with highly repetitive results. The range present in the parentheses may be used for efficiency estimation using the respective templates. BEWARE: All the template concentrations were prepared from serial dilutions of highly pure substrates. In order to have optimal results, dilutions should be freshly prepared with a crowding solution containing a background of unrelated DNA or RNA population (carrier RNA or salmon sperm DNA) to avoid specific substrate degradation and/or binding to the tube surface that may affect very low copy dilutions (less than 10 copies/µl). i***

***The cDNA template was prepared by using the Engineered M-MLV Reverse Transcriptase Basic Kit (Cat No: RN012S) in a standard endpoint reaction, containing 1 µg of HeLa RNA and oligodT(18).***

****** If using lower reaction volumes, scale all components proportionally. Reaction volumes < 10 µl are not recommended. Lower reaction volumes decrease the signal intensity.***

Prepare a master-mix calculating for "1.05 * Number of reactions needed". E.g. If 10 reactions are needed, prepare a master mix for 1.05 * 10 = 10.5 reactions. Round up to the closest higher integer. For the particular example you should round up to 11. Even if the result is 10.3 one should round up to 11. Frequent pipette service (cleaning, check in scale) by the user according to the manufacturer's instructions, is necessary to conform with the rule of 1.05.

**2.** Gently mix by inverting the vial for 4-6 times, without creating bubbles (**DO NOT VORTEX**). Bubbles may interfere with fluorescence detection. Spin down for 20 sec at 2000 g if necessary.

***Always include positive and negative control reactions. A proposed order of template addition after aliquoting is to: a) add the negative control templates and close the caps, b) add the unknown sample templates and close the caps and finally c) add the positive control reactions' templates.***

**3.** Place the reactions in the instrument and run the appropriate program according to the manufacturer's instruction.

## Three-step PCR Program

| Cycles | Duration | Temperature (°C) |
|---|---|---|
| 1 | 15 minutes | 95 |
| 40 | 15-30 seconds | 95 |
| | 30 seconds | 55-65 |
| | 30 seconds | 72 |

## Two-step PCR Program

| Cycles | Duration | Temperature (°C) |
|---|---|---|
| 1 | 15 minutes | 95 |
| 40 | 15-30 seconds | 95 |
| | 30 seconds | 55-65 |



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**Table 2. Three-step and Two-step qPCR programs.**

**Initial Heat activation for 15 min is a very essential step!**

**REDUCING TIME OF INITIAL INCUBATION/ACTIVATION AT 95°C MAY AFFECT EFFICIENCY!**

Extension time should be 50 sec/kbp of amplicon size,



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