# Ultra SYBR Green qPCR Master Mix (2X, with ROX I)

**Cat. #:** W2601-1 (1 mL); W2601-5 (5 mL)

**Storage**: Store at -20℃ for up to 1 year and avoid freeze-thaw cycles. Stored at 4℃ if it is frequent used within short time.

**Platform:** ABI Prism7500/7500 Fast, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000

## Product Components

| Cat. No. | Kit Size | 2x Ultra SYBR Green qPCR Master Mix (with ROX I) | RNase-Free Water |
|---|---|---|---|
| W2601-1 | 50 µL volume x 40 reactions, | 1 mL | 1 mL |
| W2601-5 | 50 µL volume x 200 reactions | 1 mL x 5 | 5 mL |

## Application

- Gene expression analysis
- Microarray result validation

## Product Description (This product is for research use only.)

UltraSYBR Mixture (With ROX) provides a convenient real-time PCR specific reagent by intercalater method using SYBR Green I for detection. It is a 2x concentrated premix of GoldStar Taq DNA polymerase, dNTPs, $Mg^{2+}$, SYBR Green I dye (detection), ROX reference dye and PCR buffer components. The product utilizes an enzyme for hot start, GoldStar Taq DNA polymerase, which confers a significant reduction in non-specific PCR amplification. As the enzyme buffer system is optimized for real time PCR, the product offers high amplification efficiency and high detection sensitivity in real-time PCR.

In light of the fact that the qPCR instruments can vary from user to user, Cowin offers the UltraSYBR Mixture in a range of formulations, each of which has been carefully optimized to confer the best performance according to the make and model of a qPCR machine. Please use the following table as a guide for selecting the UltraSYBR Mixture that will be most compatible with your choice of a particular instrument/model.

## Key features

- Easy-to-use 2X premix reagent including SYBR Green I
- Hot start PCR enzyme enables high efficiency and sensitivity in real-time PCR (qPCR)
- Accurate detection and quantification of target gene through real time PCR.

## Precautions before Use

- Prior to use, make sure the reagent is evenly mixed by inverting the bottle several times without creating bubbles and briefly centrifuge the tube before use.
- Store at 2-8°C to avoid repeated freeze-thaw cycles if it is frequent used within short time.
- This product contains SYBR Green I and ROX Reference Dye. Avoid exposing to strong light when preparing the reaction mixture.
- Use fresh disposable tips to avoid contamination between samples when preparing or dispensing reaction mixtures.

## Protocol

The following example is applied to conventional real-time PCR reaction system. The reaction conditions can be optimized according to the template, primer structure. This protocol is for a reaction size of approximately 50 µL. The reaction size may be adjusted as desired.

1. Prepare the PCR mixture shown below

| Reagent | 50 µL PCR reaction | Final Concentration |
|---|---|---|
| 2x Ultra SYBR Green qPCR Master Mix (with ROX I) | 25 µL | 1x |
| Forward Primer, 10 µM | 1 µL | 0.2 µM |
| Reverse Primer, 10 µM | 1 µL | 0.2 µM |
| Template DNA | 2 µL | - |
| RNase-Free Water | Up to 50 µL | - |

**Note:**

1) A final primer concentration of 0.2 µM is likely to yield good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 µM.
2) It is preferable to use 10-100 ng genomic DNA or 1-10 ng cDNA as template. The optimal quantity varies depending on the number of target copies in the template solution. Make serial dilutions to determine the appropriate amount.
3) If cDNA (RT reaction mixture) is used as template, the template volume should be no more than 10% of the PCR mixture.

2. Briefly centrifuge reaction tubes then set them for real-time PCR reaction.

This protocol is set according to ABI 7500 Real-Time PCR instrument. Two-step PCR reaction procedure is recommended. Try this protocol first and optimize PCR conditions as necessary.

**Please notice that the initial denaturation step prior to PCR should be at 95℃ for 10 min)**

Two-step PCR protocol

| Procedure | Temperature | Time | |
|---|---|---|---|
| Initial denaturation | 95℃ | 10 min | |
| Denaturation | 95℃ | 15 s | 35-40 cycles |
| Annealing / Extension | 60℃ | 1 min | |
| Melting curve analysis | | | |
| | 95℃ | 15 s | |
| | 60℃ | 1 min | |
| | 95℃ | 15 s | |
| | 60℃ | 15 s | |

**Note:**

1) This product utilizes Goldstar Taq polymerase, which is an enzyme for hot start PCR. Initial denaturation step prior to PCR should be at 95℃ for 10 min
2) This protocol is set according to ABI 7500 Real-Time PCR, melting curve analysis protocol should be adjusted according to the qPCR instrument.
3) Please try annealing of two-step PCR at 60℃ for 30 seconds at first. The temperature should be optimized within the range of 60 - 64℃ if optimization is required.
4) Perform a three-step PCR when using primers with low Tm values. The annealing temperature for three-step PCR should be optimized within the range of 56-64℃.

-- The end --