2X qPCR Master Mix, SYBR, ROX

#GCR-51S **200ul**

Store at -20^{O}C and Dark

Spin tubes briefly before use

2X qPCR Master Mix, SYBR:

The SYBR Green qPCR Master Mix is a ready to use premix of the reaction components that includes buffer, thermostable DNA polymerase, nucleotide mix and SYBR green (except primers, template and water) to perform real-time PCR. Direct detection of PCR product is monitored by measuring the increase in fluorescence caused by the binding of SYBR Green dye to double-stranded (ds) DNA.

delTaq DNA polymerase in combination with an optimized buffer ensures PCR specificity and sensitivity. PCR enhancer cocktail is added in the 2X reaction mix for better and robust performance of the PCR cycle. The polymerase and the buffer composition present in the master mix, ensures amplification from high complexity of the template. delTaq is a cold sensitive DNA polymerase that ensures no activity at room temperature during reaction set up. The master mix in real-time PCR ensures reproducible, sensitive and specific quantification of genomic, plasmid, viral, and cDNA templates. 2xqPCR Master Mixes are compatible with most real-time thermal cyclers.

Passive reference dye:

ROX Passive Reference Dye is provided additionally with the 2xqPCR master mix. ROX is an inert dye, it's fluorescence does not change during the reaction. ROX is added to quantitative, real-time PCR reactions to normalize the well-to-well loading differences that may occur due to artifacts such as pipetting errors or instrument limitations. ROX is provided in a specially formulated buffer for higher stability and sensitivity. ROX should be freshly diluted every time in nuclease-free water prior setting up the reaction. For best results, it is recommended to use freshly diluted ROX each time (if required in lower concentration). ROX provided with the 2xqPCR master mix is 5µM. ROX requirement varies from instruments to instrument. Kindly go through the following chart.

ROX Final Concentration (Instrument-specific):

ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne Plus, Step One:

- Amount per 40µl reaction: 4.0µl
- Final ROX Concentration: 500nM
- Dilution Factor: 10X

ABI 7500, ABI 7500 Fast and QuantStudio™ :

- Amount per 40 µl reaction: 0.4 µl
- Final ROX Concentration: 50 nM
- Dilution Factor: 100X.

Important:

If ROX is required to be diluted, prepare fresh dilution in Nuclease- free Water (provided). ROX diluted in aqueous solution could not be stored.

Highlights

• **Specificity—** Deltaq DNA Polymerase and the optimized buffer composition with the PCR enhancer eliminate non-specific amplification and formation of primer dimmers.

• **Sensitivity—**detects low copy number targets

• **Wide linear range—**accurate quantification across 9 orders of magnitude

• **Reproducibility and convenience—**ready-to-use 2x master mix

Applications

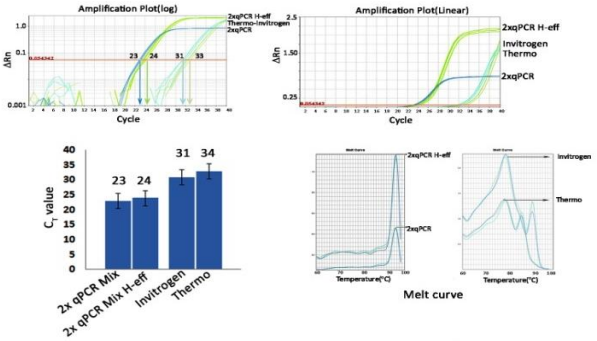
- **Gene expression**
- **Copy number detection**
- **Genotyping**
- **Pathogen detection**

Contents:

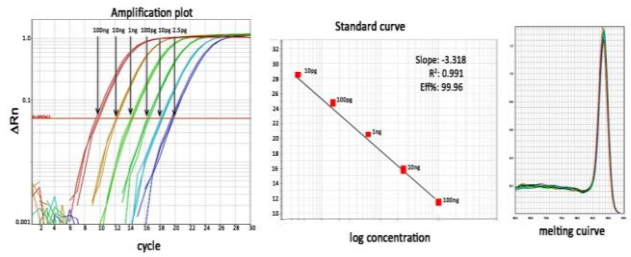
2X SYBR Green qPCR master mix: 1x200ul(sufficient for 50 x 40µl reactions) 10X ROX:1x 40ul Nuclease-free Water: 1 X 1ml

Detection capacity of the 2X qPCR master mix:

2x qPCR master mix can detect as low as single copy of template.



Comparative analysis with other vendors : 400 fg of λDNA was used to amplify a 300 bp region using 2X qPCR mix(GCR-51) and 2x H-eff qPCR mix(GCR-61). Comparitive analysis with same amount of target-primer combination showed higher C_T value with Thermo 2X DyNAmo Color Flash (F-416) and Invitrogen Power SYBR Green PCR Master Mix(4367659) (amplification plots and C_T value bar diagram). Melt curve shows specific single product with GCC 2x qPCR mix and GCC 2x qPCR H-eff mix.



Detection capability as low as 1 copy of target: 10 fold serial dilution of human genomic DNA was used as template for amplification with β-actin specific primer. Sensitivity was detected upto 1 copy (2.5pg) of template (amplification plot). Amplification on serial dilution of template shows linearity of amplification on wide range of target (Standard curve). Melting curve shows no non specific amplification..

Comparative assay:

2x qPCR master mix shows higher amplification efficiency than other commercially available master mixes, thus provides lower C_T values than others for same target.

Reaction set up for 40µl volume:

2X qPCR master mix	**20 µl**
Forward primer (10µM)	0.25 µl
Reverse primer (10µM)	0.25 µl
Template	x µl
10X ROX	4 µl
Nuclease free water	Volume makeup to 40 µl
Total volume	40 µl

Cycle parameters:

2X qPCR master mix works equally well with 2 step PCR cycle and 3 step PCR cycle. No change in C_T value was detected between 2-step and 3-step thermal reaction cycle.