

Product Overview

This is 2X concentrated solution for PCR reaction which contains dNTPs, HotStart Taq DNA Polymerase, Mg²⁺, SYBR GREEN I and other critical reaction components. It doesn't contain primers and DNA template. qPCR Mastermix is designed for routine Real Time PCR and 2nd Strand Synthesis from cDNA. You can expect the amplification upto 5KB.

Catalog Details

R2220 5 mL
R2221 20 mL

Storage

- Store the mastermix at - 20 °C when arrived. (Avoid exposure to bright light)
- Store the mastermix at 4°C for 1 month for regular usage. (Avoid exposure to bright light)

ROX Compatibility

- **High ROX Instruments** - Use the vial ROX Reference Dye (H) for instruments like Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, StepOne, StepOnePlus and other similar instruments which require high ROX
- **LOW ROX Instruments** - Use the vial ROX Reference Dye (L) for instruments like Applied Biosystems 7500, 7500 Fast Real time systems, Stratagene, QuantStudio Systems and other similar instruments which require low ROX
- **NO ROX Instruments** - Qiagen Rotor Gene, Roche LifeCycler, Biorad CFX96, CFX 384, Eppendorf MasterCycler and other similar instruments would not require ROX.

qPCR Protocol

Components	Example for 20µL reaction	Final Concentration
Template DNA/cDNA	1 µL	<100ng CDNA , <250ng of genomic DNA
Forward Primer (10µM)	0.8 µL	0.1 - 1µM
Reverse Primer (10µM)	0.8 µL	0.1 - 1µM
ROX Reference Dye (H) OR (L) OR NO ROX	0.4 µL	High - 500nM; Low - 50nM ; No ROX
qPCR MasterMix (2X)	10 µL	1X
Nuclease Free Water	Upto 20 µL	

- Total volume of cDNA templates should not be more than 10% v/v of total reaction volume.

The denaturation process is to ensure complete denaturation of the target DNA at 95°C. *This step also activates Taq DNA Polymerase which is otherwise inactive due to the binding of Anti-Taq Antibody.* The standard steps for thermal cyclers are tabulated below with optimum temperature, time, and number of cycles. Generally, 25 – 45 cycles yield sufficient product.

Step	Temperature	Time	Cycle
Initial denaturation	95 °C	1-2 minutes	1
Denaturation	95 °C	5-10s	40
Annealing */Extension	60 °C to 65°C	20 - 30s	
Melt Analysis	65°C to 95°C	variable	

Critical Note

cDNA quality depends on the initial RNA template used. Few desired genes might have very low or very high transcripts based on the cell's growth conditions. Users can empirically choose 25 to 45 cycles in the PCR step to obtain desired amplification. For very low copy transcripts use 40 cycles and for a high copy transcripts you can use 30 cycles.

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in $\geq 95\%$ purity.
2. **Performance testing:** In a 20 μ L reaction, 10 μ L of mastermix was used to amplify 0.1ng of DNA template (λ) of various sizes (100bp, 300bp, 450bp) with appropriate primers. PCR was run with 30 cycles resulted in a single product confirmed by melt curve analysis and also same sample was re-confirmed on 1% agarose gel electrophoresis with EtBr.
3. **Nuclease tests:** No contamination of endo or exonucleases were detected.

Any Technical Help ?

Please write to us at info@dxbidt.com. Response can be expected within 24Hrs. Our technical team shall be happy to assist you all the time.

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- dNTPs and rNTPs

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