Differentiating Research

Product Details

POLARIS™ SYBR Mastermix(2X)

Contains SYBR GREEN I

Product Overview

This is 2X concentrated solution for PCR reaction which contains dNTPs, HotStart Taq DNA Polymerase, Mg+2, SYBR GREEN I and an optimized reaction buffer. Polaris Mastermix is designed for routine Real Time PCR and gene expression analysis from cDNA. Non specificity is significantly reduced due to the competent buffer system and Antibody hotstart Taq DNA Polymerase which results in early Ct values. Both high and low ROX are provided as passive reference dyes.

Catalog Details

R2220	5 mL
R2221	20 mL

Storage

- Store the mastermix at - 20 ℃ when arrived. (Avoid exposure to bright light)

Mastermix Compatibility

- High ROX Instruments - Use the vial **ROX Reference Dye (HIGH)** 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 (LOW)** 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. However, if your master-mix already contains ROXs then also you can continue using the same without any trouble.

qPCR Protocol

Components	Example for 20µL reaction	Final Concentration
Template DNA/cDNA	1 µL	<100ng
Forward Primer (10µM)	0.8 µL	0.1 - 1µM
Reverse Primer (10µM)	0.8 µL	0.1 - 1µM
High ROX/ Low ROX	0.4 µL	1X
Polaris 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℃. ***This step also activates Taq DNA Polymerase which is otherwise inactive due to the binding of Anti-Taq Antibody***. The standard steps for thermal cycler 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 ℃	2 minutes	1
Denaturation	95 ℃	5-10s	40
Annealing */Extension	60 ℃ to 65℃	20 - 30s	
Melt Analysis	65℃ to 95℃	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 ≥ 95% purity.
2. **Performance testing:** In a 20µL reaction, 10µL of mastermix was used to amplify 0.1ng of DNA from OKRA leaves (200bp) with appropriate primers. PCR was run with 35 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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