

## Product Overview

An essential aspect of setting up multiplex reactions is the accommodation of multiple primer and probe sets within the final reaction volume. Polaris, with its 4X concentration, provides users with the utmost flexibility to tailor their reactions precisely to their needs.

This is 4X concentrated solution for PCR reaction which contains dNTPs, HotStart Taq DNA Polymerase, Mg+2, and an optimized reaction buffer. The mastermix was designed for probe based PCR applications. The probe is designed to bind to the target DNA during the PCR reaction and emit fluorescence as it is amplified, allowing for accurate quantification of the initial amount of target DNA in the sample.

## Catalog Details

R2224	5 mL
R2225	20 mL

## Storage

- Store the mastermix at - 20 °C when arrived. (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
Forward Primer/s(10µM)	0.5 µL	0.1 - 1µM
Reverse Primer/s( 10µM)	0.5 µL	0.1 - 1µM
ROX Reference Dye (H) OR (L) OR NO ROX	0.4 µL	High - 500nM; Low - 50nM ; No ROX
PROBES	variable	0.1-0.5µM
Polaris-AMP Mastermix(4X)	5 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 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 °C	1-2 minutes	1
Denaturation	95 °C	5-10s	30-40
Annealing */Extension	60 °C to 65°C	20 - 30s	
(Read Signal)			

## 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

- Purity:** SDS Page analysis with Coomassie Blue Staining resulted in  $\geq 95\%$  purity for enzymes.
- Performance testing:** In a 20 $\mu$ L reaction, 10 $\mu$ L of mastermix was used to amplify 0.1ng of DNA template (lambda) of various sizes ( 100bp, 300bp, 450bb) 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.
- Nuclease tests:** No contamination of endo or exonucleases were detected.

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