Imagen ® One-Step Probe RT-PCR Fastermix

Cat. No IPRF 001

Description

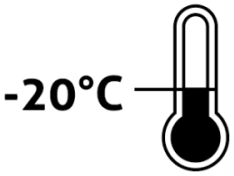
Imagen ® One-Step Probe RT-PCR Fastermix is a ready-to-use and that can perform cDNA synthesis and PCR reaction all at once, 2 X concentration premix including a novel Fast Hotstart Taq DNA polymerase, Fast Reverse transcriptase, optimized reaction buffer and nucleotides with combines accurate using sequence-specific fluorogenic probe chemistries including hydrolysis probes (ex. TagMan, Molecular beacon, etc…). This formulation is engineered for superior efficiency in the conversion of sample RNA into cDNA and sensitive amplification.

Contents

The Fastermix is supplied as a ready-to-use 2x reaction mix. The formulation contains reverse transcriptase, DNA polymerase enzyme, dNTPs, $MgCl_2$, reaction enhancers, and stabilizers.

Storage

- ✓ -20 °C
- ✓ Protected from light
- ✓ Avoid repeated freezing and throwing



Reaction Mix Thawing and Handling

The Imagen ® One-Step Probe RT-PCR Fastermix is delivered in a 2x ready-to-use format. To use the mix, thaw the vial on ice to 4 °C.

Please completely mix the vial and briefly centrifuge to ensure all components are at the bottom of the tube. Store on ice protected from light until ready to use. If using automated liquid handling, let sit at ambient temperature for 10 min to further reduce the viscosity.

Application

- ✓ One step qRT-PCR based on Specific Probes.
- ✓ Detection and Quantification of DNA and cDNA targets.
- ✓ Gene Expression.
- ✓ Fast qPCR platforms.
- ✓ High Throughput Applications.

Recommend for Assay Design and Optimization

- ◆ For best qPCR efficiency, design assays targeting an amplicon size of 70 - 150 bp.
- ◆ The Imagen ® One-Step Probe RT-PCR Fastermix cycling protocols have been optimized for assays with a predicted primer melting temperature (Tm) of 58 - 62 °C.
- ◆ The probe's Tm should be 8 – 10 °C higher than the calculated primer Tm.

Prepare the RT–PCR Reaction Mix

1. Mix the Imagen ® One-Step Probe RT-PCR Fastermix thoroughly but gently until it's completely homogenous.
2. Prepare the RT–PCR Reaction Mix for the number of reactions required as shown in table below and plus 10% overage.

Reagent	Volume (ul)	Final conc.
2 x One-Step Probe RT-PCR Fastermix	12.5	1x
Forward Primer	Variable	300 - 600 nM
Reverse Primer	Variable	300 - 600 nM
Fluorogenic Probe	Variable	200 - 200 nM
RNA Template	2 - 4	100 ng - 10 pg
Nuclease-free water	Up to 25	-
Final volume	**25**	-

3. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube. (*Use good pipetting practice to ensure assay precision and accuracy of dispensing.)
4. Add RNA (and nuclease-free water, if needed) to the PCR tubes or wells containing the reaction mix, seal tubes or wells with flat caps or optically transparent film, and gently vortex to ensure thorough mixing of the reaction components.
5. Program the thermal cycling protocol on the real-time PCR instrument.

Step		Temp. ℃	Time	Cycles
Reverse transcription		45 - 48	5- 10 min	1
DNA polymerase activation and template denaturation		95	1 min	1
Amplification	Template denaturation	95	3 sec	40
	Annealing / Extension and plate read	58 - 62	≥ 20 sec	

6. Load the PCR tubes or plates onto the real-time PCR instrument and start the RT-qPCR run program.
7. When thermal cycling is complete, perform data according to the instructions in the instrument-specific software.