# Imagen ® Multiplex TaqMan qPCR Premix

**Cat. No**

## Description

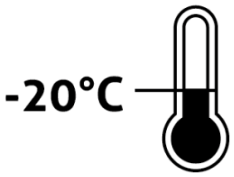
**Imagen ® Multiplex TaqMan qPCR Premix** is a ready-to-use, 2X concentrated premix including a novel Hotstart Taq DNA polymerase、optimized reaction buffer and nucleotides with Rox reference dye and combines accurate using sequence-specific fluorogenic probe chemistries including hydrolysis probes (ex. TaqMan、Molecular beacon,etc…) which provides good specificity and amplification efficiency for real-time PCR.

## Contents

The **Imagen ® Multiplex TaqMan qPCR Premix** is supplied as a ready-to-use 2x reaction mix. The formulation contains, Hotstart Taq DNA polymerase, dNTPs, $MgCl_2$, reaction enhancers, and stabilizers.

## Storage

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




## Reaction Mix Thawing and Handling

**Imagen ® Multiplex TaqMan qPCR Premix** 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

- ✓ Quantitative real-time PCR
- ✓ Gene expression analysis
- ✓ Low copy gene detection

## Prepare the qPCR Reaction Mix

1. Mix the **Imagen ® Multiplex TaqMan qPCR Premix** thoroughly but gently until it's completely
2. homogenous.
   Prepare the qPCR Reaction Mix for the number of reactions required as shown in table below and plus 10% overage.

| Reagent | Volume (ul) | Final conc. |
|---|---|---|
| Imagen ® Multiplex TaqMan qPCR Premix | 12.5 | 1x |
| Forward Primer A (10 uM) | 0.75 | 300 - 600 nM |
| Reverse Primer A (10 uM) | 0.75 | 300 - 600 nM |
| Fluorogenic Probe A (10 uM) | 0.5 | 200 nM |
| Forward Primer B (10 uM) | 0.75 | 300 - 600 nM |
| Reverse Primer B (10 uM) | 0.75 | 300 - 600 nM |
| Fluorogenic Probe B (10 uM) | 0.5 | 200 nM |
| DNA Template | 2 | 100 ng - 10 pg |
| Nuclease-free water | 6.5 | - |
| **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 DNA (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 |
|---|---|---|---|---|
| DNA polymerase activation and template denaturation Amplification | | 95℃ | 10 min | 1 |
| Amplification | Template denaturation | 95℃ | 15 sec | 35-40 |
| | Annealing / Extension and plate read | 58 - 65℃ | 30 sec © Data acquisition | |

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