

TaqMan 2x RT- PCR Master Mix - 100 Reactions**Product Insert**

Product # 28341

Description:

Norgen's TaqMan 2x RT-PCR Master Mix is a ready-to-use solution that contains a PCR internal control which can be detected by HEX/VIC channel in a real-time PCR machine. By detecting the internal control users can validate the RNA template quality, thereby preventing any false negatives in the RT-PCR results. The user needs only to add template, target TaqMan primer/probe mix and water to set up the TaqMan real-time RT-PCR.

PCR Control:

TaqMan 2x RT-PCR Master Mix contains PCR control primers/probe (HEX/VIC) and PCR control template. The PCR control reaction in the TaqMan 2x RT-PCR Master Mix is optimized to not interfere with target amplification. The fluorescence of the target probe should not be HEX/VIC.

Advantages:

- Convenience and time savings
- Cost efficient
- High sensitivity
- Avoid false negatives due to template quality

**Applications:**

- Routine TaqMan RT-PCR
- Sensitive detection with internal control

Reagents supplied:

- TaqMan 2x RT-PCR Master Mix (3 Vials, 100 Reactions)

Storage Conditions:

TaqMan 2x RT-PCR Master Mix should be stored at -20°C. For everyday use aliquots can be stored at 4°C for up to 3 months. The TaqMan 2x RT-PCR Master Mix is stable for multiple freeze-thaw cycles. When stored at the proper temperature this reagent is stable for at least 1 year.

Precautions and Disclaimers:

This product is designed for research purposes only. It is not intended for human or diagnostic use.

Tips for Performing PCR Reactions:

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) is a powerful method used to amplify specific RNA transcripts using multiple cycles of a two-part process: Part I, Reverse transcription and Part II, PCR cycle containing denaturation, annealing, and extension steps. Successful RT-PCR relies on various factors, and it is important to keep a number of points in mind when performing RT-PCR:

1. Using high quality, purified RNA templates greatly enhances the success of RT-PCR.
2. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
3. There should be designated RNase-free solutions, tips, tubes, pipettes, etc. for RT-PCR only.
4. Optimize the template amount: up to 1 µg RNA.

Procedure

Reaction Setup Table

| TaqMan RT-PCR Reaction Mixture | Single 20 μ L Rxn | 10 Rxn + 1 Rxn ** |
|--------------------------------|-----------------------|---------------------|
| TaqMan 2x RT-PCR Master Mix | 10 μ L | 110 μ L |
| Target Primer/Probe Mix* | 2 μ L | 22 μ L |
| Template RNA | 2 - 5 μ L | 2 - 5 μ L / rxn |
| Nuclease-Free Water | Up to 20 μ L | Up to 220 μ L |

* Suggested concentration of primer (F and R) and probe is 2.5 μ M. The fluorescence of the target probe should not be HEX/VIC.

** Experienced User Protocol for Reaction Preparation for Multiple Samples

1. Dispense 10 μ L of TaqMan 2X RT-PCR Master Mix into the PCR tube.
2. Add template RNA (0.5 – 1 μ g) and Target Primer/Probe Mix to the PCR tube as shown in the Reaction Setup Table.
3. Add nuclease-free water to bring the total volume to 20 μ L.
4. Mix the PCR mixture thoroughly and spin down briefly.
5. Place the PCR tubes into the PCR machine and carry out the RT-PCR according to the Suggested One-Step TaqMan RT-PCR Program shown in the table below.

Suggested One-Step TaqMan RT-PCR Cycle Conditions

| PCR Cycle | Step | Temperature | Duration |
|---------------|-----------------------|-------------|----------|
| Cycle 1 | Reverse Transcription | 50°C | 30 min |
| Cycle 2 | Initial Denaturation | 95°C | 3 min |
| Cycle 3 (40X) | Denaturation | 95°C | 15 sec |
| | Annealing / Extension | 60°C | 30 sec |

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.