

2X One-Step RT-PCR Master Mix
(1 x 100 Reactions)
Product # 28113**Product Insert****Description:**

Norgen's 2X One-Step RT-PCR Master Mix is a ready-to-use solution that contains components required for RT-PCR amplification of RNA templates. The mix includes M-MuLV reverse transcriptase, Taq DNA polymerase, dNTPs, reaction buffer, MgCl₂, KCl, and a PCR enhancer/stabilizer. The user needs only to add template, the primer set and water to the Master Mix to set up the RT-PCR reaction.

Taq Source:

An *E. coli* strain with a cloned *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1.

RT Source:

An *E. coli* strain with a cloned reverse transcriptase gene from M-MuLV.

Specifications:

- Convenience and time savings
- Cost efficient
- High sensitivity and specificity
- Good reproducibility

Applications:

- Routine and direct RT-PCR amplification of RNA templates
- Multiple band detection or genotyping

Reagents supplied:

- 2X RT-PCR Master Mix (1 Vial, 100 Reactions). Sufficient reagent for 100 x 20 µL reactions

Storage Conditions:

2X RT-PCR Master Mix should be stored at -20°C. For everyday use an aliquot can be stored at 4°C for up to 3 months. Repeated freeze-thaw cycles are not recommended. 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.

- Primers should have a melting temperature of approximately 60°C. Use primer design software.
- Design primers to generate an amplicon up to 1.5 kb
- To avoid amplification of the genomic copy of the target, either treat the RNA template with DNase I or design the primer set to be flanking an intron.

Procedure

Reaction Setup Table

| RT-PCR Reaction Mixture | 20 µL Rxn | 50 µL Rxn |
|--------------------------------|-------------|-------------|
| 2X One-Step RT-PCR Master Mix | 10 µL | 25 µL |
| Template RNA (300 fg - 300 ng) | 1-2 µL | 1-2 µL |
| Primer F (2 - 10 µM) | 1 µL | 1 µL |
| Primer R (2 - 10 µM) | 1 µL | 1 µL |
| Nuclease-Free Water | Up to 20 µL | Up to 50 µL |

- Dispense either 10 µL or 25 µL of 2X One-Step RT-PCR Master Mix into the PCR tube according to the desired final volume of the RT-PCR reaction (see Reaction Setup Table above).
- Add template RNA (300 fg to 300 ng of total RNA, poly A RNA, or in vitro transcribed RNA) and both forward and reverse primers (enough for a final concentration of 100-500 nM) to the PCR tube as shown in the Reaction Setup Table.
- Add nuclease-free water to bring the total volume to either 20 µL or 50 µL.
- Mix the RT-PCR mixture thoroughly and spin down briefly.
- Place the PCR tubes into the PCR machine and carry out the RT-PCR according to the suggested RT-Cycle Conditions in the tables below

Suggested PCR Cycle Conditions

| PCR Cycle Step | Temperature | Time | No. of Cycles |
|-----------------------|-------------|-----------------|---------------|
| Reverse Transcription | 50°C | 30 min | 1 |
| Initial Denaturation | 94-95°C | 5 min | 1 |
| Denaturation | 94-95°C | 15-30 sec | 30-40 |
| Annealing | 55-60°C | 15-30 sec | |
| Extension | 65-72°C | 1 minute per kb | |
| Final Extension | 72°C | 5 min | 1 |
| Hold | 4-10°C | Indefinitely | 1 |

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.