BIO-RAD

Reliance One-Step Multiplex Supermix

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EMPOWER YOUR PCR

Catalog #	Description
12010176	**Reliance One-Step Multiplex Supermix**, 1 ml (1 x 1 ml vial), 200 x 20 µl reactions
12010220	**Reliance One-Step Multiplex Supermix**, 5 ml (5 x 1 ml vials), 1,000 x 20 µl reactions
12010221	**Reliance One-Step Multiplex Supermix**, 10 ml (2 x 5 ml vials), 2,000 x 20 µl reactions

For research purposes only.

Introduction

Reliance One-Step Multiplex Supermix is a ready-to-use 4x reaction mix optimized for reverse transcription quantitative PCR (RT-qPCR) multiplex probe-based detection. It contains the proprietary Reliance Reverse Transcriptase Enzyme with an optimized blend of DNA polymerases. This supermix formulation is engineered for superior efficiency in the conversion of sample RNA into complementary DNA and sensitive multiplex amplification. It allows for amplification of up to five targets even in the presence of common PCR inhibitors. Reliance One-Step Multiplex Supermix also contains stabilizers and RNase inhibitors to prevent the degradation of RNA, allowing storage of assembled reactions at room temperature for up to 24 hours without loss of performance.

Storage and Stability

Store at –20°C, protected from light, upon receipt. Guaranteed stable for a minimum of 12 months when stored properly. The supermix will not freeze at –20°C, though gelling and precipitation may occur. This is expected and does not impact its stability or performance when used as instructed. For convenience, the supermix can be stored at 4°C for up to a week.

Kit Contents

The supermix is supplied as a ready-to-use 4x reaction mix. The formulation of the mix contains reverse transcriptase, DNA polymerase enzyme blend, dNTPs, $MgCl_2$, universal reference dyes, reaction enhancers, and stabilizers.

Instrument Compatibility

This supermix is compatible with all Bio-Rad real-time PCR systems. The mix is formulated with a blend of reference dyes, including ROX, for compatibility with all common qPCR instrument platforms, including the following instruments:

- **Applied Biosystems:** 7000, 7300, 7500, 7700, 7900HT, QuantStudio, StepOne, StepOnePlus, and ViiA 7
- **Stratagene:** Mx3000P, 3005P, and 4000
- **Eppendorf:** Mastercycler ep *realplex* 2 and 4
- **QIAGEN:** Rotor-Gene 3000, 6000, and Q
- **Roche:** LightCycler 1.0, 1.5, and 2.0, LightCycler 96 and 480

Reaction Mix Thawing and Handling

The Reliance One-Step Multiplex Supermix is viscous. It is critical to mix it thoroughly before use.

1. Let the vial sit at ambient temperature for 10 min.
2. Mix thoroughly by pulse vortexing the vial, then briefly centrifuge to collect contents at the bottom of the vial.
3. Ensure the supermix is a light pink, precipitation-free solution. Repeat step 2 as necessary until there is no precipitation remaining.
4. Store on ice, protected from light, until ready to use.

Reaction Mix Preparation and Thermal Cycling Protocol

1. Prepare the RT-qPCR reactions according to Table 1. Prepare the reaction mix with enough overage, typically 10%, to account for volume loss from liquid handling.

Table 1. Reaction setup.*

Component	Volume per 20 µl Reaction	Volume per 10 µl Reaction	Final Concentration
4x Reliance One-Step Multiplex Supermix	5 µl	2.5 µl	1x
Forward and reverse primers**	Variable	Variable	100–900 nM each
Fluorogenic probe(s)**	Variable	Variable	150–250 nM each
RNA template (add at step 3)	Variable	Variable	1 pg–1 µg
Nuclease-free water	To 20 µl	To 10 µl	—
Total reaction mix volume	**20 µl**	**10 µl**	**—**

* Scale all components proportionally according to sample number and reaction volumes.

** For PrimePCR Probe Assays, add 1 µl of assay solution per 20 µl reaction and 0.5 µl per 10 µl reaction.

2. Mix the reaction mix thoroughly to ensure homogeneity and dispense equal aliquots into each PCR tube or into the wells of a PCR plate. Use good pipetting practice to ensure assay precision and accuracy of dispensing.
3. Add RNA (and nuclease-free water, if needed) to the PCR tubes or wells containing the reaction mix (Table 1), seal tubes or wells with flat caps or optically transparent film, and gently vortex to ensure thorough mixing of the reaction components.

4. Spin the tubes or plate to remove any air bubbles and collect the reaction mixture in the vessel bottoms. Assembled reactions should be stable for 24 hr at ambient temperature (25°C) without significant loss of assay performance.
5. Program the thermal cycling protocol on the real-time PCR instrument according to Table 2.

Table 2. Thermal cycling protocol.*

Step		Temperature, °C	Time	Number of Cycles
Reverse transcription		50	10 min	1
DNA polymerase activation and template denaturation		95	10 min	1
Amplification	Template denaturation	95	10 sec	35–40
	Annealing/extension and plate read	60	30 sec	

* Fast cycling protocols can be used on fast thermal cyclers with additional optimization recommended for best performance.

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 analysis in CFX Manager Software or CFX Maestro Software or according to the instructions in the instrument-specific software.

Recommendations for Multiplex Assay Design and Optimization

- For best qPCR efficiency, design assays targeting an amplicon size of 70–150 bp
- The Reliance One-Step Multiplex Supermix cycling protocols have been optimized for assays with a predicted primer melting temperature (T_m) of 60°C and designed using the open source Primer3, Primer3Plus, or Primer-BLAST programs with their default settings. The predicted T_m of oligonucleotides will vary between programs. If primers are designed using other programs, adjust the temperature accordingly
- The probe's T_m should be 8–10°C higher than the calculated primer T_m. In a multiplex reaction, applying the brighter fluorophores to the lower-expressing targets and the less bright fluorophores to the higher-expressing targets can help in visualizing data and minimizing the opportunity for signal cross talk between channels
- The Bio-Rad PrimePCR Probe Assays for Gene Expression are expertly designed and can be used for multiplexing with minimal cross-reactivity

No-RT Control Reaction

To determine that no genomic DNA is being codetected with the target RNA molecule, an inactivated RT control reaction can be performed using a heat-kill method. To perform this, place an aliquot of the Reliance One-Step Multiplex Supermix into a thermal cycler or dry bath and hold at 95°C for 1 min to eliminate the activity of the RT enzyme while leaving the DNA polymerase unaffected. This solution lacking active RT enzyme can be used to prepare the no-RT control reaction according to the instructions in Table 1. If no genomic DNA is present, the reaction should yield a negative amplification result.

Quality Control

Reliance One-Step Multiplex Supermix demonstrates high RT-qPCR efficiency and linear resolution over a wide linear dynamic range. Detection sensitivity is validated in a multiplex reaction detecting as little as ten copies per reaction with RNA standard material normalized by Droplet Digital PCR. Reliance One-Step Multiplex Supermix is manufactured under ISO 13485:2016 to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

Related Products

Catalog #	Description
1725080	**SingleShot Cell Lysis Kit**, 100 x 50 µl reactions
1725081	**SingleShot Cell Lysis Kit**, 500 x 50 µl reactions

Visit **bio-rad.com/PrimePCR** for expertly predesigned probe assays for gene expression.

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