TruQuant Taqman Multiplex Master Mix

FB05002010 100 reactions 1 mL

FB05002050 500 reactions 5 mL

Store the master mix at -25°C to -15°C. After first use, it can be stored at 2°C to 8°C. Use before expiration date. Protect from light.

TruQuant Taqman Multiplex Master Mix (2X) can be used with TaqMan probes and primers for DNA virus research. It is optimized for pathogen detection and gene expression analysis.

Kit Contents

Reagents	100 Reactions	500 Reactions
2X TruQuant Taqman Multiplex Master Mix	1 mL	5 mL
50X ROX Reference Dye (50X)	40 µL	200 µL

Reaction Setup

The recommended reaction volume for thermal cycling protocol is up to 30 µL. The recommended final concentration of the primers is 400 - 900 nm and that of the the probes is 100 - 250 nm. Add 1 pg to 100 ng of nucleic acid sample to each reaction.

Component	Volume per Reaction for 96-Well (0.2 mL) Plate
2X TruQuant TaqMan Master Mix	10 µL
ROX Reference Dye (optional)	0.4 µL
Target-specific Primers and Probes	1 µL
Nucleic Acid Sample	varies
Water, nuclease-free	to 20 µL

1. Prepare the reaction premix by adding each component in proportion to the required number of reactions and reserve 10% margin for each component to avoid pipetting losses.
2. Mix all components thoroughly and then centrifuge quickly.
3. Transfer the reaction mixture into the wells of the reaction plate.
4. Cover the reaction plate with a chemical film.
5. Centrifuge the reaction plate quickly.

Cycling Conditions

TruQuant Taqman Multiplex Master Mix is compatible with fast or standard cycling protocols. For QuantStudio™ 5 Real-Time PCR System, 96-well, 0.2 mL, use "fast" mode. For 7500 Real-Time PCR System, use standard cycling mode.

Real-Time PCR System	Enzyme Activation	PCR (40 cycles)	
	Hold at 95 °C	Denaturation at 95 °C	Annealing / Extension at 60 °C
7500 Real-Time PCR System	2 min	3 sec	30 sec
QuantStudio™ 5 Real-Time PCR System, fast cycling mode	2 min	1 sec	20 sec

Note: The UDG in this master mix is a heat-labile UDG. The digestion of dU-containing DNA occurs while preparing the qPCR plate, so it is not necessary to have a UDG step in the thermal protocol. The heat-labile UDG is inactivated during the initial 95°C / 2 min step.

Result Analysis

1. Review amplification curves and make modifications as needed.
2. In the reaction well table or results list, review the Ct values for each well and each replicate group.
3. (For standard curve experiments) Review the relevant parameters of the standard curve:
   a. Slope
   b. Amplification efficiency
   c. R^2 value
   d. Y-axis intercept
   e. Ct values
   f. Outliers

Baseline and Threshold Overview

The baseline and threshold of the amplification plot can be set automatically or manually using the real-time PCR system software.

Baseline: The initial PCR cycles where the fluorescence signal shows slight variations.

Threshold: The intersection of the amplification plot that determines the Ct value of the real-time PCR assay kit. The threshold is set above the background signal during the exponential growth phase of the amplification curve.