TruQuant SYBR Green Master Mix

FB05001010 100 reactions 1 mL
FB05001050 500 reactions 5 mL
Store the master mix at -25°C to -15°C. Protect from light.

TruQuant SYBR Green Master Mix is a 2X ready-to-use premix. This product is designed for dye-based quantitative amplification of DNA targets in cDNA and gDNA templates via real-time PCR. Suitable for various challenging templates and targets and applicable for various primer melting temperatures (Tm) ranging from 55°C to 65°C.

Reaction Setup

Use 1-10 ng cDNA or 10-100 ng gDNA per reaction. Reactions smaller than 10 µL are not recommended. The recommended final concentration of the primers is 400 nM with a Tm of 60°C. Recommend 4 replicates for each sample.

Component	10 µL Reaction	20 µL Reaction	Final Concentration
2X TruQuant SYBR Green Master Mix	5 µL	10 µL	1X
Forward and Reverse Primers	0.5 µL	1 µL	400 nM
Template DNA	1 µL	2 µL	0.5 ng/µL
Water, nuclease-free	to 10 µL	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.

Prepared qPCR plates can be stored at room temperature for up to 8 hours if protected from light.

For Research Use Only. Not for use in diagnostic procedures.

Instrument: 7500 Fast System, 7500 System, 7900HT Fast System, 7900HT System, QuantStudio 3, QuantStudio 5.

Cycling Conditions

1. Set Thermal Cycling program

Step	Temp.	Fast Reaction Duration	Standard Reaction Duration	Cycles
Enzyme Activation	95°C	2 min	2 min	1
Denature	95°C	5 sec	15 sec	40
Anneal and Extend	60°C	30 sec	60 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.

2. Set Melt Curve program

Step	Fast Ramp Rate (°C/sec)	Standard Ramp Rate (°C/sec)	Temp.	Time
1	1.99	1.6	95°C	15 sec
2	1.77	1.6	60°C	1 min
3 (melting curve)	0.075	0.075	95°C	15 sec

For the 7500 Real-Time PCR System, use the default ramp rate.

3. Set Options

- Experiment Type: Standard Curve
- Reagent: SYBR™ Green Reagent
- Reporter Dye: SYBR™ Green
- Quencher Dye: None
- Passive reference dye: ROX™
- Ramp speed: Standard or fast (choose the same setting as in step 2)
- Melt curve ramp increment: Continuous

Result Analysis

1. Observe the amplification curve.
2. Use instrument software to set appropriate baseline and threshold.
3. Check for non-specific amplification using the melting curve.
4. Perform relative or absolute quantification.