

## ab286848 – Taqman Master Mix

For TaqMan probe-based real-time PCR analysis of DNA samples.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab286848>

### Storage and Stability

On receipt product should be stored at -20°C, protected from light. After each experiment, the leftover mix (completely thawed and thoroughly homogenized) can be stored at 4°C for 3 months. This product is stable for 1 year from the date of shipping.

### Materials Supplied

Item	Quantity	Storage Condition
Taqman Master Mix	4 x 1.25 mL (20 µL)	+4 °C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- PCR Tubes
- QPCR Instrument
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Template DNA

### Reagent Preparation

1. Aliquot reagents to avoid contamination and to avoid repeated freeze-thaw cycles.
2. The Taqman Master Mix components are light sensitive and therefore, avoid prolonged exposure to direct light.
3. Ideally, start the PCR as soon as the reaction mixture is prepared. If not, then make sure that the reaction mixture is kept chilled till starting up the PCR.
4. Primer concentration should not be high, a concentration of 100 nM to 300 nM of each primer usually gives the best results.
5. A very effective way to get tight Ct among replicates is to reduce pipetting error, this can be achieved by preparing amplicon specific pre-mix, using repeating pipet, and keeping pipetting volume in manufacture suggested range.
6. For optimal results, it is recommended that the primers are 18-22 nucleotides in length with a Tm of 58°C-60°C and the size of target is about 100-250 bp.

### QPCR Protocol

1. Thaw the Taqman 2X Master Mix, template DNA, primers, and nuclease-free water on ice. Mix each solution thoroughly.
2. Prepare a reaction Master Mix as follows:

Components	Volume (20 µL)	Volume (25 µL)	Volume (50 µL)	Final Concentration
Taqman Master Mix	10 µL	12.5 µL	25 µL	1X
Forward Primer	Varies	Varies	Varies	100 – 500 nM
Reverse Primer	Varies	Varies	Varies	100 – 500 nM
Template DNA	Varies	Varies	Varies	≤10 ng/reaction
TaqMan Probe	Varies	Varies	Varies	100 – 300 nM
Water, Nuclease-free	To 20 µL	To 25 µL	To 50 µL	-
<b>Total Volume</b>	<b>20 µL</b>	<b>25 µL</b>	<b>50 µL</b>	-

3. Perform QPCR reactions using the following 3-step cycling program:

Step	Temperature	Duration (Standard)	Duration (Fast)	Cycle(s)
Enzyme Activation	95 °C	10 minutes	10 minutes	1
Denaturation	95 °C	15 seconds	3 seconds	40
Annealing/Extension	60 °C	60 seconds	30 seconds	40

### Technical Support

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