

# HELINI

## Human TUBB3

### Real-time PCR Kit

Instructions for use



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HELINI Biomolecules, Chennai, INDIA

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**Intended Use**

The HELINI human TUBB3 Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of TUBB3 (tubulin beta 3 class III) specific RNA.

**Kit components**

Components	Volume Per reaction	Number of vials	Volume Per vials
One step RT-PCR Master Mix	8 $\mu$ l	4	200 $\mu$ l
RT-Taq enzyme mix	2 $\mu$ l	4	50 $\mu$ l
TUBB3 Primer Probe Mix [TUBB3 PP mix]	5 $\mu$ l	4	125 $\mu$ l
Positive control Mix [Lyophilized]	10 $\mu$ l	1	250 $\mu$ l
Water, PCR grade		1	4ml

**Positive control supplied in lyophilized form. Please refer Page.9 for dilution procedure.**

**Storage**

- The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

**Material and instruments required**

- Real-time PCR instrument having FAM channel
- Automatic Nucleic acid extraction system or spin column based purification kit for the purification of nucleic acids
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Centrifuge with a rotor for PCR strips/tubes and 96 well plates
- Optical cap qPCR tubes or strips or 96 wells
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

*[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.]*

**Product Use Limitations**

- All reagents may exclusively be used in molecular research.
- The product is to be used by personnel specially instructed and trained in Molecular research.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (RNase/RNase) contamination of the specimens and the components of the kit.
- Always use RNase/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

**Technical Assistance**

For technical assistance and more information, please contact;

0091-9382810333

0091-44-244490433

helinibiomolecules@gmail.com

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## Dilution before Use

### Positive control

**Caution:** High Positive template, Use dedicated pipette and tips box and unique area other than the regular qPCR Mix preparation cabinet or place.

1. Centrifuge the vials at 10000rpm for 3min.
2. Add **245µl of PCR grade water**, close the vial.
3. Incubate at room temperature for 10min.
4. Gentle vortex for 5 seconds OR invert mix several times.
5. Incubate 5min at room temperature.
6. Gentle pipette mix up and down for 5 seconds.
7. Centrifuge at 1000 - 2000rpm for 5seconds. [Pulse spin]
8. Now, it is ready for use. After use, store at -20C.

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## Product description

HELINI human TUBB3 Real-time PCR kit constitutes a ready-to-use system for the detection of TUBB3 specific RNA using reverse transcriptase polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification and for the direct detection of the specific amplicon in FAM channel. In addition, External qualitative positive control is supplied to assist the experiment.

### Specificity

TUBB3 Primer and probe have been designed for the specific and exclusive *in vitro* detection of TUBB3 specific RNA (tubulin beta 3 class III). The target sequence in this kit has 100% homology with a broad range of relevant reference sequences based on a comprehensive bioinformatics analysis.

### Dynamic linear range

The linear range was evaluated by analyzing a logarithmic dilution series of pDNA concentrations ranging from 1.00E+09 to 1.00E+00 copies/µl. At least six replicates per dilution were analyzed. The linear range is 1.00E+09 to 1.00E+00 copies/µl.

### Analytical Sensitivity

The analytical sensitivity is defined as the concentration of RNA molecules (copies/ $\mu$ l) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified TUBB3 specific pDNA from 0.001copies to 10copies/ $\mu$ l in triplicates. Under optimal PCR conditions, the analytical sensitivity is 1 copy per micro liter.

#### Note:

### RNA Purification

Purified RNA is the starting material for the Real-time PCR assay. The quality of the purified RNA has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for RNA purification is compatible with real-time PCR technology.

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the RNA.

### Detection Protocol

#### Things to do before starting

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

In a sterile 1.5ml micro-centrifuge tube, add	
Components	Volume per reaction
One step RT-PCR Master Mix	8 $\mu$ l
RT-Taq enzyme mix	2 $\mu$ l
TUBB3 PP Mix	5 $\mu$ l
	<b>15<math>\mu</math>l</b>
Close the tube and gently invert mix well, spin down briefly Dispense <b>15<math>\mu</math>l</b> into PCR tubes, and then thoroughly mix the sample RNA by pipetting up and down.	
<b>Purified RNA</b>	<b>10<math>\mu</math>l</b>
Final reaction volume	25 $\mu$ l

**Negative Control setup [NTC]**

Add 10µl of PCR grade water.

**Positive Control setup**

Add 10µl of Positive control

Centrifuge PCR vials briefly before placing into thermal cycler.

*[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]*

**Programming Thermal cycler**

<b>Sample volume</b>	25µl
<b>Fluorescence Dyes</b>	FAM
<b>Passive reference</b>	None
<b>Ramping rate</b>	Default

**Thermal Profile**

	<b>Step</b>	<b>Time</b>	<b>Temp</b>
	Reverse transcriptase	20min	50°C
	Taq enzyme activation / Hold	15min	95°C
<b>40 cycles</b>	Denaturation	20sec	95°C
	Annealing/Data collection*	20sec	60°C
	Extension	20sec	72°C

<b>Data collection/Acquisition</b>	<b>Targets</b>
FAM	TUBB3

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**Reading the graph:****Negative and Positive control validation**

Select the NTC and Positive control and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The Positive Control must be amplified before 28 cycles.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

**Test Sample status**

In FAM channel, select test sample well, analyze the graph/amplification.

**Limitations**

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the HELINI TUBB3 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.



### Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI TUBB3 Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

### Explanations of symbols



Research use only



Catalogue number



Pack size – number of tests



Manufacturer

Manufactured by

**HELINI Biomolecules,**  
Ohmlina, 26, 2<sup>nd</sup> Avenue,  
Khuthubi Complex, Vettuvankeni,  
Chennai 600115, Tamilnadu, INDIA  
www.helini.in  
info@helini.in  
helinibiomolecules@gmail.com  
+91-44-24490433 / +91-9382810333

