

## MBT128 Hi-Quanti One Step RT-PCR Kit (Real-Time PCR SYBr Based)

### Description

HiMedia's Hi-Quanti One Step RT-PCR Kit (Real-Time PCR SYBr Based) is a convenient one-step formulation, for both cDNA synthesis and PCR amplification in a single tube using gene-specific primers from either total RNA or mRNA. The advantages of one-step real-time RT-PCR is that it is quicker to set up and involves less handling of samples, thereby reducing pipetting errors, contamination and other sources of error. This makes processing multiple RNA samples easy when you are amplifying only a few genes of interest. The MMuLV Reverse Transcriptase enzyme included in the kit consists of a proprietary buffer system that has been optimized for reverse transcription and PCR and includes  $Mg^{+2}$  and Deoxy ribonucleotide triphosphates (dNTPs).

### Product packing

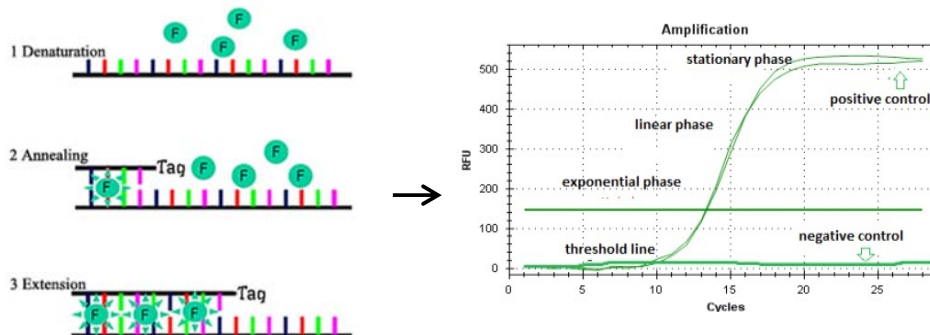
HiMedia's Hi-Quanti One Step RT-PCR Kit (Real-Time SYBr PCR Based) is available in following packs:

Product Name	Product Code	Kit Packing
Hi-Quanti One Step RT-PCR Real Kit	MBT128-10R	10 reactions
	MBT128-25R	25 reactions
	MBT128-50R	50 reactions
	MBT128-2x50R	2 x 50 reactions

### Features

- Efficient, fast RT-PCR in a single tube
- Works efficiently with GC-rich and highly structured RNA templates
- Quick and easy reaction preparation

Real-time PCR also called quantitative PCR (qPCR) or kinetic PCR, is a laboratory technique based on the principle of PCR. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. The presence of SYBr Green Dye a dsDNA-binding dye in the Hi-SYBr Master Mix allows for simplified assay design without the need for additional fluorescent probes and enables assay verification using a melt-curve analysis.



The SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

**Hi-Quanti One Step RT-PCR Real Time Kit is provided with**

Components	Product Code	Reagents provided for			
		10R	25R	50R	2x50R
Hi-SYBr Master Mix (with Taq Polymerase)	MBT074	150 µL	400 µL	800 µL	2 x 800 µL
One Step RT Enzyme Mix	DS0286	15 µL	40 µL	80 µL	2 x 80 µL
Molecular Biology Grade Water	ML065	500 µL	1 mL	2 mL	2 x 2mL

**Storage and Stability**

Store the Hi-Quanti One Step RT-PCR Kit (Real-time PCR Based) at –20°C. When stored under recommended conditions, the kit components are stable for 1 year.

**Procedure**

1. Add the reagents as follows

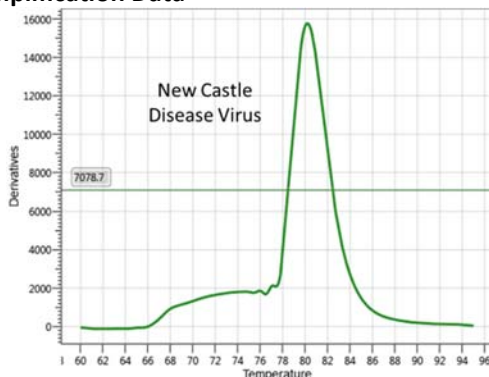
Ingredients	Volume per reaction (µL)
Hi-SYBr Master Mix (with Taq Polymerase)	10
One Step RT Enzyme Mix	1
Gene Specific Forward Primer (10 µM)	1
Gene Specific Reverse Primer (10 µM)	1
Molecular Biology Grade Water (ML065)	Up to 20
RNA template	X

- Gently mix and ensure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed, depending on the thermal cycler used, if necessary.
- Place the reaction in the preheated thermal cycler programmed as described below. Program the thermal cycler so that cDNA synthesis is followed immediately with PCR amplification automatically.
- Collect the data and analyze the results.

**Recommended PCR program**

- |                      |                                                                        |             |
|----------------------|------------------------------------------------------------------------|-------------|
| cDNA Synthesis       | : 50°C for 15 minutes                                                  | } 40 cycles |
| Initial denaturation | : 95°C for 2 minutes 30 seconds                                        |             |
| Denaturation         | : 94°C for 30 seconds                                                  |             |
| Annealing            | : 55-65°C for 30 seconds (Plate Read)                                  |             |
| Melt curve stage     | : Melt Curve Analysis as per HiMedia’s Insta Q96 Real Time PCR Machine |             |
|                      | 95°C for 15 seconds                                                    |             |
|                      | 60°C for 1 minute                                                      |             |
|                      | 95°C for 15 seconds                                                    |             |
|                      | Increment for 0.5°C                                                    |             |
|                      | Holding time for 10 seconds                                            |             |

**Amplification Data**



Sr. No.	Sample	C <sub>t</sub> value	T <sub>m</sub> (°C)
1	Positive control	12.56	80.9
2	Negative control	N/A	No T <sub>m</sub>

Image representing real-time amplification data of New Castle Disease samples with C<sub>t</sub> values (provided in table)

## Quality control

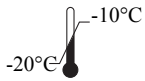
Each lot of Hi-Quanti One Step RT-PCR Kit (Real-Time PCR SYBr Based) is functionally tested for performance; free of endo, exo- deoxyribonuclease, ribonuclease and nicking activities.

## Storage and shelf-life

Hi-Quanti One Step RT-PCR Kit (Real-Time PCR SYBr Based) should be stored at -20°C and is stable for 1 year when stored under proper conditions.

## Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at [mb@himedialabs.com](mailto:mb@himedialabs.com).



Storage temperature



Do not use if package is damaged



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## Disclaimer :

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