

MBT190

Hi-PCR® One Step Mastermix

Product Name	Product Code	Kit Packing
Hi-PCR® One Step Mastermix	MBT190-10R	10 reactions
	MBT190-25R	25 reactions
	MBT190-50R	50 reactions

Description:

Hi-PCR® One Step Mastermix is specially designed for qPCRs that directly use RNA as templates. By using this a master mix system, the reverse transcription and PCR can be finished in one tube, significantly reducing pipetting procedures and the risk of contamination. The reverse transcriptase and hot-start Taq DNA Polymerase included in this product enables high-sensitive total RNA detection (as little as 1 pg). The 2X One-step Buffer Mix contains an optimized buffer and dNTP/dUTP mix and is suitable for high-sensitive detection systems based on fluorescence labelled probes. This mastermix system allows multiplex probe based detection.

Hi-PCR® One Step Mastermix includes below components:

Components	Product Code	Reagents provided for 10R (reactions)*	Reagents provided for 25R (reactions)*	Reagents provided for 50R (reactions)*
2X One-step Buffer Mix	DS1086	110 µl	275 µl	550 µl
One-step RT enzyme Mix	DS1087	11 µl	27.5 µl	55 µl
Molecular Biology Grade Water for PCR	ML065	100 µl	250 µl	500 µl

*For 20µl reaction

Storage and Stability:

Store the Hi-PCR® One Step Mastermix at -20°C in a constant temperature freezer. When stored under recommended conditions, the kit components are stable for 1 year.

General Instructions:

To avoid nuclease contamination, please keep the experiment area clean, wear clean gloves and masks, and use nuclease-free tubes and tips.

Procedure:

1) Add the reagents as follows:

Ingredients	Product Code	Volume per 20µl reaction mix
2X One-step Buffer Mix	DS1086	10 µl
One-step RT enzyme Mix	DS1087	1 µl
Gene Specific Forward Primer (10 µM)*	Not provided	0.4 µl
Gene Specific Reverse Primer (10 µM)*	Not provided	0.4 µl
10µM Probe [#]	Not provided	0.2 µl
Template RNA	Not provided	Total RNA: 1 pg-1 µg
Molecular Biology Grade Water for PCR	ML065	Upto 20 µl

* The final concentration of primer is usually 0.2 µM, and if necessary, it can be adjusted between 0.1 µM and 1.0 µM.

The final concentration of probe can be adjusted between 50 nM and 250 nM.

NOTE: The mastermix is compatible with all Real-Time PCR machines. The appropriate detection channel should be selected as per the fluorophore used in the assay.

2) Gently mix and ensure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

3) Place the sample in a qPCR instrument and run the following program for One Step qRT-PCR:

Standard PCR program:

- Reverse Transcription : 50°C* for 15 minutes
- Initial denaturation : 95°C for 30 seconds
- Cycling Parameters (No. of cycles: 45)
 - Denaturation : 95°C for 10 seconds
 - Annealing : 60°C for 30 seconds[#]

Fast PCR program:

- Reverse Transcription : 50°C for 5 minutes
- Initial denaturation : 95°C for 30 seconds
- Cycling Parameters (No. of cycles: 45)
 - Denaturation : 95°C for 5 seconds
 - Annealing : 60°C for 20 seconds^{**}

*For templates with complex secondary structure or high GC-content, the temperature can be increased to 55 °C, which will improve the sensitivity and performance.

The extension time varies between different qPCR instruments used.

** Please check the compatibility of fast program for the qPCR instrument.

Quality control:

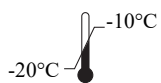
Each lot of Hi-PCR® One Step Mastermix is functionally tested in qRT-PCR.

Troubleshooting Guide:

Sr.No.	Problem	Possible cause	Possible solution
1	No amplification product	RNase contamination	Maintain aseptic conditions
		Not enough starting template RNA	Increase the concentration of template RNA
		RNA has been damaged or degraded	Replace RNA if necessary
		RT inhibitors are present in RNA	Remove inhibitors in the RNA preparation by an additional 70% ethanol wash. Note: Inhibitors of RT include SDS, EDTA, guanidium salts, formamide, sodium phosphate and spermidine
2	Low specificity	Reaction conditions not optimal	• Optimize magnesium concentration • Optimize the primer • Optimize the annealing temperature and extension time
3	Non-specific amplification	Contamination by genomic DNA	• Pretreat RNA with DNase I
		Nonspecific annealing of primers	• Vary the annealing temperature • Optimize the magnesium concentration for each template and primer combination

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.



Storage temperature



Do not use if package is damaged



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