Super 4X One-Step Multiplex Master Mix

Cat. No./Spec.		
Cat. No.	V5012-A	V5012-B
20-μl reaction Nos.	200 rxns	5,000 rxns

Components

Component	V5012-A	V5012-B
Super 4X One-Step Multiplex Master Mix *	1 ml × 1	25 ml × 1

* Contains Reverse Transcriptase, RNase inhibitor, Heat-labile UDG and hot-start Taq DNA Polymerase, dNTPs including dUTP and buffer components.

Storage

This reagent should be kept at -15~-25°C.

Description

The Super 4X One-Step Multiplex Master Mix is used to perform one-step multiplex real-time PCR applications with any gene-specific primer and probe sets, and is suitable for both RNA and DNA targets. Dual hot-start reverse transcriptase and DNA polymerase can complete more than 10-plex highly sensitive and specific quantitative PCR in a single system. The master mix is supplied at a 4X concentration that allows to input more sample into each reaction, increasing sensitivity even in low-volume reactions.

Protocol

1. Preparation of Reaction System

1.1 Prepare the reaction system by referring to the table below. Thaw all reagents on ice. When multiple reaction wells are prepared, 10% margin should be reserved for each component to avoid pipetting loss.

1.2 Cover the reaction plate with optical film. Mix well by flipping and then centrifuge.

Fast reaction system:

Component	20-μl rxn	Final conc.
Super 4X One-Step Multiplex Master Mix	5 μl	1X
Primer-Probe mix	1 μl	Primer: 400-900 nM Probe: 100-250 nM
Sample*	Adjust as needed	1 pg-100 ng
RT-PCR Grade Water	Adjust as needed	–
Total volume	20 μl	

Standard reaction system:

Component	50-μl rxn	Final conc.
Super 4X One-Step Multiplex Master Mix	12.5 μl	1X
Primer-Probe mix	2.5 μl	Primer: 400-900 nM Probe: 100-250 nM
Sample*	Adjust as needed	1 pg-100 ng
RT-PCR Grade Water	Adjust as needed	–
Total volume	50 μl	

* DNA or RNA samples are acceptable. Reverse transcription does not affect the DNA samples.

2. Preform RT-qPCR using the following thermal cycling condition

Fast reaction system:

Step	Stage	Cycle No.	Temperature	Time
Reverse transcription *	1	1	55°C	20 min
Polymerase activation	2	1	95°C	2 min
Amplification	3	45	95°C	3 sec
			60°C	30 sec

Standard reaction system:

Step	Stage	Cycle No.	Temperature	Time
Reverse transcription *	1	1	55°C	20 min
Polymerase activation	2	1	95°C	2 min
Amplification	3	45	95°C	15 sec
			60°C	60 sec

* The recommended temperature for the Hotstart reverse transcription is 55 °C and the time is more than 15 min.

3. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information. In general, data analysis mainly includes:

1. Observe the amplification curve and set it according to needs, such as:

a. set appropriate baselines and threshold lines

b. remove some typical outliers from the analysis

2. Observe whether there is any difference in Ct value between the multiple wells;

3. For absolute quantification, observe the slope, amplification efficiency, R^2 value, intercept, Ct value and outliers of the standard curve.

Product Use Limitations

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.