DSPath™ 4X One-Step Multiplex Master Mix (High ROX+)

Cat. No.: V5005H, V5006H

Components

Component	V5005H (200 rxns, 20 μl/rxn)	V5006H (5000 rxns, 20 μl/rxn)
DSPath™ 4X Multiplex Master Mix (High ROX+) *	1 ml × 1	25 ml × 1

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

Storage

This reagent should be kept at -15~-25°C and protect from light.

Description

The DSPath™ 4X One-Step Multiplex Master Mix (High ROX+) is used to perform one-step multiplex real-time PCR applications with any gene-specific primer and probe sets in quantitative instruments that require high concentrations of ROX, and is suitable for both RNA and DNA targets. This master mix is formulated with optimized buffer components to accommodate multiplex amplification of up to four RNA or DNA target sequences in a single reaction. 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	Volume	Final concentration
DSPath™ 4X Multiplex Master Mix (High ROX+)	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	Volume	Final concentration
DSPath™ 4X Multiplex Master Mix (High ROX+)	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 *	10 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 *	10 min
Polymerase activation	2	1	95°C	2 min
Amplification	3	45	95°C	15 sec
			60°C	60 sec

* The temperature can be adjusted between 48°C and 55°C.

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.