Ver.EN20230920

Hifair™ Advanced One Step RT-qPCR SYBR Green Kit

Product description

Hifair™ Advanced One Step RT-qPCR SYBR Green Kit is a kit for fluorescence quantification based on SYBR Green I dye. Using gene-specific primers, reverse transcription and qPCR reactions are completed in one tube, eliminating the need for repeated cap-opening and pipetting operations, greatly improving assay efficiency and reducing the risk of contamination. For RNA samples, the kit employs heat-resistant Hifair™ V Reverse Transcriptase for efficient cDNA synthesis and UNICON™ HotStart Taq DNA Polymerase for quantitative amplification. Under the optimized buffer system, the sensitivity of the kit can be as high as 0.1 pg for highly expressed targets and as high as 1 pg for moderately expressed targets, and the kit is also suitable for amplification and quantification of DNA samples. The kit is suitable for the amplification and quantification of DNA samples. It enables the sensitive detection and quantification of nucleic acids from different plant and animal samples, cells and microorganisms.

Specifications

Cat.No.	11175ES20 / 11175ES70
Size	20 T/200 T

Components

Components No.	Name	11175ES20	11175ES70
11175-A	2× Hifair™ Advanced SG Buffer	250 µL	2×1.25 mL
11175-B	Hifair™ Advanced UH Enzyme Mix	20 µL	200 µL
11175-C	RNase Free H_2O	250 µL	2×1.25 mL

Storage

This product should be stored at -25~-15°C away from light for 1 year.

Instructions

1. Reaction system configuration*****

Components	Volume (µL)****	Volume (µL)	Final concentration
2× Hifair™ Advanced SG Buffer	12.5	25	1×
Hifair™ Advanced UH Enzyme Mix	1	2	-
Forward Primer (10 µmol/L)**	0.5	1	0.2 µmol/L
Reverse Primer (10 µmol/L)**	0.5	1	0.2 µmol/L
RNA***	X	X	-
RNase Free H_2O	to 25	to 50	-

** The final primer concentration was 0.2 µmol/L, which could also be adjusted between 0.1 and 1 µmol/L as appropriate.

*** The reagent is extremely sensitive, with Total RNA in the range of 1 pg-1 µg, and testing of human samples showed an optimal input of 1 pg-100 ng, controlling for an overall Ct value in the range of 15-30 as appropriate.

**** It is recommended to use 20 μL or 50 μL to ensure the validity and reproducibility of target gene amplification.

***** Please prepare in the ultra-clean bench and use nuclease residue-free tips and reaction tubes; tips with filter cartridges are recommended. Avoid cross contamination and aerosol contamination.

2) Reaction program

Cycle step	Temp.	Time	Cycles
Reverse transcription	50°C*	6 min	1
Initial denaturation	95°C	5 min	1
Amplification reaction	95°C	15 sec	40
	60°C**	30 sec	
Melting curve stage	Instrument Defaults		1

*The reverse transcription temperature can be selected between 50-55°C according to the experimental needs. For DNA samples, the reverse transcription process can be omitted.

** In special cases the annealing/extension temperature can be adjusted according to the primer Tm value, 60°C is recommended.

3) Applicable Model

Instrument models that do not require Rox calibration:

Bio-Rad: CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4;

Eppendorf: Mastercycler ep realplex, realplex 2 s;

Qiagen: Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000;

Roche Applied Science: LightCycler 480, LightCycler2.0, Lightcycler 96;

Thermo Scientific: PikoReal Cycler; Cepheid: SmartCycler; Illumina: Eco qPCR;

Low Rox applicable models:

ABI 7500, 7500 Fast, ViiA7, QuantStudio 3 and 5, QuantStudio 6, 7, 12k Flex;

Stratagene MX3000P, MX3005P, MX4000P;

High Rox applicable models:

ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus.

Notes

1. This product is for research use only.
2. Please operate with lab coats and disposable gloves，for your safety.