

Hifair™ Universal Advanced Multiplex One Step RT-qPCR Mix (UDG Plus)

Product description

Hifair™ Universal Advanced Multiplex One-Step RT-qPCR Mix (UDG Plus) is a ready-to-use master mix designed for multiplex quantitative PCR directly from RNA templates. The reverse transcription and qPCR amplification occur in a single tube, streamlining workflow and minimizing the risk of cross-contamination.

This reagent is optimized for multiplex RNA amplification with the following key features:

- Broad compatibility: Delivers robust performance across diverse primer-probe sets.
- Rapid cycling: Supports fast protocols with total run times ≤ 35 minutes.
- Enhanced specificity and sensitivity: The proprietary buffer contains components that suppress non-specific amplification and boost efficiency in multiplex reactions.
- dUTP/UDG anti-contamination system: Effectively prevents carryover contamination from amplicons via uracil-DNA glycosylase (UDG).
- High tolerance to complex samples: Compatible with direct RT-qPCR from swab specimens (e.g., oral swabs) without prior RNA purification.

Specifications

Cat NO.	16930ES60 / 16930ES80 / 16930ES92
Size	100 T / 1,000 T / 10,000 T

Components

Components No.	Name	16930ES60(100 T)	16930ES80(1,000 T)	16930ES92(10,000 T)
16930-A	5×Hifair™ MP Buffer	500 μ L	5 mL	50 mL
16930-B	Hifair™ Enzyme Mix	100 μ L	1 mL	10 mL
16930-C	GC-Enhancer	500 μ L	5 mL	50 mL

[Note]: 5× Hifair™ MP Buffer is an abbreviation for 5× Hifair™ Universal Advanced Multiplex One-Step RT-qPCR Buffer.

Hifair™ Enzyme Mix contains thermostable reverse transcriptase, HotStart Taq DNA polymerase, RNase inhibitor, and UDGase.

Storage

This product should be stored at -25°C ~ -15°C for 1 year when stored as directed.

Instructions

1. Reaction Setup (25 μ L Example)

Cat NO.	Component	Volume (μ L)	Final Concentration
16930-A	5× Hifair™ MP Buffer	5	1×
16930-B	Hifair™ Enzyme Mix	1	—
16930-C	GC-Enhancer	0–5	—

Cat NO.	Component	Volume (μL)	Final Concentration
/	Primer Mix (10 μM each)	0.4 per primer	0.16 μM each
/	Probe Mix (10 μM each)	0.2 per probe	0.08 μM each
/	RNA Template	1–5	—
/	Nuclease-free H ₂ O	to 25	—

[Note]: Important Notes on Reaction Setup:

Mix all components thoroughly before use; avoid vigorous pipetting to prevent bubble formation.

1. GC-Enhancer: A multifunctional additive that improves amplification of high-GC (>70%) or problematic templates by reducing primer-dimers and non-specific products. It may affect primer-template binding stability; include only when necessary.
2. Primer concentration: Final concentration per primer can be optimized within 0.1–1.0 μM.
3. Probe concentration: Final concentration per probe can be adjusted between 50–300 nM, depending on signal intensity and multiplex complexity.
4. Template dilution: Due to high assay sensitivity, dilute RNA to achieve Ct values between 20–35 for optimal quantification.
5. Reaction volume: Use 25 μL or 50 μL total volume to ensure reproducibility and amplification efficiency.
6. Preparation environment: Assemble reactions in a PCR workstation using RNase-free, aerosol-resistant filtered tips and certified nuclease-free tubes to prevent contamination.

2. Recommended Thermal Cycling Programs

2.1 Standard Program

Step	Temperature	Time	Cycles
Reverse Transcription	50°C ^a	15 min	1
Initial Denaturation	95°C	5 min	1
Amplification	95°C	15 sec	45
	60°C ^b	30 sec ^c	

[Note]:

- a. Reverse transcription can also be performed at 55°C for 10–15 min.
- b. Annealing/extension temperature should be adjusted based on primer T_m values.
- c. Fluorescence acquisition settings depend on the instrument; set to the shortest recommended read time.

2.2 Fast Program

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	2 min	1
Initial Denaturation	95°C	30 sec	1
Amplification	95°C	5 sec	40
	60°C	15 sec ^d	

[Note]: d: Confirm instrument compatibility with fast cycling protocols via pilot experiments.

3. Compatible Real-Time PCR Instruments

Applied Biosystems: 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne Plus™, 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 & 5, QuantStudio™ 6/7/12K Flex

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, LightCycler 2.0, LightCycler 96

Agilent (Stratagene): MX3000P™, MX3005P™, MX4000P™

Thermo Scientific: PikoReal Cyclor

Cepheid: SmartCycler

Illumina: Eco qPCR

SLAN: SLAN-96S, SLAN-96P, SLAN-48S

Notes

1. Always use RNase-free consumables throughout the workflow.
2. Wear a lab coat and disposable gloves for personal protection.