

Hifair™ AdvanceFast 1st Strand cDNA Synthesis Kit (No Dye)

Product description

Hifair™ AdvanceFast 1st Strand cDNA Synthesis Kit (No Dye) is a rapid reverse transcription kit developed based on the Hifair™ III 1st Strand cDNA Synthesis Kit (No Dye), suitable for PCR amplification and RT-qPCR experiments. Compared to the Hifair™ III 1st Strand cDNA Synthesis Kit (No Dye), it boasts stable detection rates, specificity, and yield assurance, with the total reverse transcription time potentially reduced to less than 6 minutes, significantly shortening experimental duration.

The kit includes gDNA Digester Mix, which can eliminate genomic DNA contamination remaining in the RNA template, ensuring more reliable subsequent results. The kit provides two types of cDNA synthesis primers: Random Primers N6 and Oligo (dT)₁₈. Users can choose between Random Primers N6, Oligo (dT)₁₈, or Gene Specific Primers as reverse transcription primers according to their needs. The synthesized single-stranded cDNA product can be directly used for subsequent PCR or qPCR reactions.

Specifications

Cat.No.	11150ES10 / 11150ES60
Size	10 T/100 T

Components

Components No.	Name	11150ES10	11150ES60
11150-A	4 × Hifair™ AdvanceFast SuperMix (No Dye)	50 µL	500 µL
11150-B	5 × gDNA Digester Mix	20 µL	200 µL
11150-C	Random Primers (50 µmol/L)	20 µL	200 µL
11150-D	Oligo d(T) ₁₈ Primers (50 µmol/L)	20 µL	200 µL
11150-E	RNase-free Water	200 µL	2 × 1 mL

Storage

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

Instructions

1. If the experiment requires the removal of residual genomic DNA

1) gDNA Digestion

In a RNase-free centrifuge tube, prepare the following mixture and gently pipette to mix well. Incubate at 42°C for 2 minutes.

Components	Volume (µL)
RNase-free Water	To 10 µL
5 × gDNA Digester Mix	2 µL
Total RNA*	10 pg-5 µg

or mRNA*	10 pg-500 ng
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* It is recommended that the input amount of Total RNA should not exceed 2 µg. If the expression level of the target gene is low, the input amount can be increased to a maximum of 5 µg Total RNA.

2) Reverse Transcription Reaction System Preparation (20 µL System)

Components	Volume (µL)
the reaction solution from the previous step	10 µL
4× Hifair™ AdvanceFast SuperMix (No Dye)	5 µL
Oligo d(T) ₁₈ Primers (50 µmol/L)**	2 µL
or Random Primers (* for qPCR) (50 µmol/L)**	or 2 µL
RNase-free Water	To 20 µL

* The amount of primers can be adjusted according to the amount of template input. If the reverse transcription product is used for qPCR experiments, Random Primers can be added in the system according to the recommended amount.

** It is recommended to first add 4× Hifair™ AdvanceFast SuperMix (No Dye), mix well, and then add the reverse transcription primer to ensure that the primer is not affected by gDNA Digester.

3) Reverse Transcription Procedure Settings

Temp.	Time
55°C*	5 min**
85°C	5 sec

* For templates with high GC content or complex templates, the reverse transcription temperature can be increased to 60°C.

** This product can synthesize cDNA sequences below 14 kb. If you need longer cDNA products, you can appropriately extend the reverse transcription time.

2. If the experiment does not require the removal of genomic DNA

1) Reverse Transcription Reaction System Preparation (20 µL System)

Components	Volume (µL)
4× Hifair™ AdvanceFast SuperMix (No Dye)	5 µL
Oligo d(T) ₁₈ Primers (50 µmol/L)**	2 µL
or Random Primers (* for qPCR) (50 µmol/L)**	or 2 µL
Total RNA*	10 pg -5 µg
or mRNA	10 pg - 500 ng
RNase-free Water	To 20 µL

* It is recommended that the input amount of Total RNA should not exceed 2 µg. If the expression level of the target gene is low, the input amount can be increased to a maximum of 5 µg Total RNA.

** The amount of primers can be adjusted according to the amount of template input. If the reverse transcription product is used for qPCR experiments, Random Primers can be added in the system according to the recommended amount.

2) Reverse Transcription Procedure Settings

Temp.	Time
55°C*	5 min**
85°C	5 sec

* For templates with high GC content or complex templates, the reverse transcription temperature can be increased to 60°C.

** This product can synthesize cDNA sequences below 14 kb. If you need longer cDNA products, you can appropriately extend the reverse transcription time.

Notes

1. All operations should be performed on ice and should be performed in a manner that avoids RNase contamination.
2. This product is for research use only.
3. Please operate with lab coats and disposable gloves, for your safety.