

Hieff NGS™ C37P3 ds-cDNA Synthesis Kit(gDNA digester plus)

13501ES

INSTRUCTIONS FOR USE

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CONTENT

PRODUCT DESCRIPTION 1

SPECIFICATIONS 1

COMPONENTS1

STORAGE 1

INSTRUCTIONS 2

NOTES1

Product description

The Hieff NGS™ C37P3 ds-cDNA Synthesis Kit (gDNA digester plus) is a cDNA synthesis kit designed for RNA sequencing library preparation on Illumina/MGI sequencing platforms. It includes gDNA removal reagents, RNA reverse transcription reagents, and ds-cDNA synthesis reagents. All reagents provided in this kit have undergone strict quality control and functional validation to ensure the stability and reproducibility of library construction to the greatest extent.

Specifications

Cat.No.	13501ES08 / 13501ES24 / 13501ES96
Size	8 T / 24 T / 96 T

Components

Components No.		Name	13501ES08	13501ES24	13501ES96
			8 T	24 T	96 T
13501-A		DNase I Buffer	16 µL	48 µL	192 µL
13501-B		DNase I Mix	16 µL	48 µL	192 µL
13501-C		1st Reaction Buffer	48 µL	144 µL	580 µL
13501-D		1st Strand Enzyme Mix	16 µL	48 µL	192 µL
13501-E		2nd Reaction Buffer	56 µL	168 µL	672 µL
13501-F		2nd Strand Enzyme Mix	24 µL	72 µL	288 µL

Storage

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

Notes

I. About Operation

1. For your safety and health, please wear a lab coat and disposable gloves during operation.
2. Thaw all kit components at room temperature before use. After thawing, invert the tubes several times to mix thoroughly, briefly centrifuge, and keep on ice until use.
3. It is recommended to perform all reaction steps in a thermal cycler with a heated lid. Preheat the thermal cycler to the required reaction temperature before use.
4. Use RNase-free consumables and regularly clean the experimental area. We recommend using ThermoFisher's RNAzap™ Efficient RNA Decontamination Spray to remove RNase contamination.
5. Improper handling of PCR products can easily generate aerosol contamination, which may compromise the accuracy of experimental results. It is recommended to enforce strict physical separation between the PCR

reaction setup area and the PCR product purification/detection area. Use pipettes dedicated to library preparation, and regularly clean each experimental area (we recommend using ThermoFisher's DNAZap™ Efficient DNA Decontamination Spray) to maintain a clean experimental environment.

6. This product is for research use only!

II. Materials Required But Not Supplied

1. DNA purification beads: Hieff NGS™ DNA Selection Beads (Yeasten Cat#12601ES), AMPure® XP Beads (A63880), or other equivalent products.

2. Library quality control: Agilent 2100 Bioanalyzer DNA 1000 Chip / High Sensitivity Chip or other equivalent products; library quantification reagents.

3. Other materials: Absolute ethanol, sterile ultrapure water, low-retention pipette tips, PCR tubes, magnetic stand, thermal cycler, etc.

Instructions

Step 1: gDNA Digestion

1. Thaw the gDNA digestion reagents at room temperature, mix by inversion, and keep on ice until use. Prepare the gDNA digestion reaction mixture according to Table 1 below.

Table 1: gDNA Digestion Reaction System

Name	Volume (μL)
DNase I Buffer	2
DNase I Mix	2
RNA	X
RNase-free H ₂ O	13-X
Total	17

2. Mix gently by pipetting up and down, and briefly centrifuge to collect the reaction mixture at the bottom of the tube.

3. Place the PCR tube in a thermal cycler and set the reaction program as shown in Table 2 to perform gDNA digestion.

Table 2: gDNA Digestion Reaction Program

Temperature	Time
Heated lid 105°C	On
37°C	15 min
94°C	7 min
4°C	Hold

Step 2: First-Strand cDNA Synthesis

1. Remove the First-Strand Synthesis Reagent from -20°C, thaw at room temperature, mix by inversion, and briefly centrifuge. Prepare the first-strand cDNA synthesis reaction mixture according to Table 3.

Table 3: First-Strand cDNA Synthesis Reaction System

Name	Volume (μL)
RNA (gDNA digester plus)	17
1st Reaction Buffer	6
1st Strand Enzyme Mix	2
Total	25

2. Mix gently by pipetting up and down, and briefly centrifuge to collect the reaction mixture at the bottom of the tube.

3. Place the PCR tube in a thermal cycler and run the first-strand cDNA synthesis program as shown in Table 4. Proceed immediately to second-strand cDNA synthesis after the reaction is complete.

Table 4: First-Strand cDNA Synthesis Reaction Program

Temperature	Time
Heated lid 105°C	On
25°C	5 min
42°C	30 min
85°C	5 min
4°C	Hold

[Note]: To shorten the experimental workflow, a rapid program may be selected: 25°C for 5 min, 42°C for 10 min, 85°C for 5 min.

Step 3: Second-Strand cDNA Synthesis

1. Remove the Second-Strand Synthesis Reagent from -20°C, thaw, and mix by inversion. Prepare the second-strand cDNA synthesis reaction mixture according to Table 5.

Table 5: Second-Strand cDNA Synthesis Reaction System

Name	Volume (μL)
1st Strand cDNA	25
2nd Reaction Buffer	7
2nd Strand Enzyme Mix	3
Total	35

2. Mix gently by pipetting up and down, and briefly centrifuge to collect the reaction mixture at the bottom of the tube.

3. Place the PCR tube in a thermal cycler and run the second-strand cDNA synthesis program as shown in Table 6.

Table 6: Second-Strand cDNA Synthesis Reaction Program

Temperature	Time
Heated lid 105°C	Off
16°C	30 min
4°C	Hold

[Note]: To shorten the experimental workflow, a rapid program may be selected: 16°C for 5 min.

Step 4: cDNA Product Purification

1. The 2nd Strand cDNA product can be directly used with the Hieff NGS™ C37P4 OnePot cDNA & gDNA Library Prep Kit for library construction, or stored at -20°C.
2. Alternatively, perform second-strand cDNA product purification (Post Ligation Clean Up):
 - 1)Preparation: Remove the Hieff NGS™ DNA Selection Beads from the refrigerator and allow them to equilibrate at room temperature for at least 30 minutes. Prepare 80% ethanol.
 - 2)Vortex or invert the beads thoroughly to ensure they are fully resuspended and mixed.
 - 3)Add 63 µL of Hieff NGS™ DNA Selection Beads (1.8×, Beads:DNA = 1.8:1) to the Adapter Ligation product. Vortex or pipette up and down to mix, and incubate at room temperature for 5 min.
 - 4)Briefly centrifuge the PCR tube and place it on a magnetic stand to separate the beads from the liquid. After the solution becomes clear (approximately 5 min), carefully remove and discard the supernatant.
 - 5)Keeping the PCR tube on the magnetic stand, add 200 µL of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 sec, then carefully remove and discard the supernatant.
 - 6)Repeat step 5) for a total of two washes.
 - 7)Keeping the PCR tube on the magnetic stand, open the cap and air-dry the beads until they just begin to crack (do not exceed 5 min).
 - 8)Remove the PCR tube from the magnetic stand, add 21 µL of ddH₂O, and vortex or gently pipette up and down to mix thoroughly. Incubate at room temperature for 5 min. Briefly centrifuge the PCR tube and place it back on the magnetic stand. After the solution becomes clear (approximately 3 min), carefully transfer 20 µL of the supernatant to a new PCR tube for subsequent reactions, or store at -20°C.

Note:

[illegible]



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