LongSeq cDNA Library Prep Kit

Cat. No. KITG-146 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit uses Oligo (dT) Primer as the primer and takes advantage of the terminal transferase activity (template-switching activity) of reverse transcriptase to add a special sequence to the 3′ end of cDNA. PCR amplification is then performed using this special sequence and the terminal portion of the Oligo (dT) Primer as primers to prepare full-length cDNA.
Form	Size 8 T / 24 T / 96 T Kit Components Oligo (dT) Primer — 8 µL (8 T); 24 µL (24 T); 96 µL (96 T) 5× RT Buffer — 32 µL (8 T); 96 µL (24 T); 384 µL (96 T) 1st Strand Enzyme Master Mix — 16 µL (8 T); 48 µL (24 T); 192 µL (96 T) TSO Primer — 8 µL (8 T); 24 µL (24 T); 96 µL (96 T) 2nd Reaction Buffer — 56 µL (8 T); 168 µL (24 T); 672 µL (96 T) 2nd Strand Enzyme Mix — 24 µL (8 T); 72 µL (24 T); 288 µL (96 T) 2× Multiplex Long PCR Master Mix — 200 µL (8 T); 600 µL (24 T); 2 × 1200 µL (96 T) PCR-F — 20 µL (8 T); 60 µL (24 T); 240 µL (96 T) PCR-R — 20 µL (8 T); 60 µL (24 T); 240 µL (96 T)
Bio-activity	Quality Control Test For the amplified and purified product, the recommended quality-control criteria before downstream library construction are: • Purity: $OD_{260}/_{280}$ = 1.7–2.0; $OD_{260}/_{230}$ = 1.0–2.2, assessed by UV-Vis spectrophotometry. • Yield: 1.5–4 µg, assessed by fluorescence-based nucleic acid quantification. • Length: assess the length distribution using an automated electrophoresis instrument. If the purified amplified product substantially deviates from these quality-control criteria—for example, obvious contamination, insufficient concentration, or an abnormally short length distribution—sequencing of the resulting library may produce insufficient data, reduced read lengths, and a lower usable data rate.

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Applications	Applications Full-length cDNA synthesis from poly(A)-tailed RNA samples for cDNA library preparation. Compatible Sample Types Poly(A)-tailed RNA samples. The recommended total RNA input is 150 ng. Not suitable for prokaryotic total RNA or degraded RNA, such as FFPE RNA.
Notes	Notes A thermal cycler with a heated lid is recommended for all reaction steps; preheat the thermal cycler to near the reaction temperature before use. PCR products are highly prone to aerosol contamination when handled improperly, which can affect the accuracy of the results. Technical Notes Suitable for full-length cDNA synthesis from poly(A)-tailed samples. The recommended total RNA input is 150 ng. If less than 150 ng total RNA is available, the input amount may be reduced; for example, when UHRR (human reference RNA) input is reduced to 50 ng, the amplification yield is approximately 1 µg. Increasing the number of PCR cycles to obtain sufficient cDNA may introduce additional nonspecific amplification and reduce the proportion of full-length cDNA. This kit is not suitable for prokaryotic total RNA or degraded RNA, such as FFPE RNA. Do not pause after first-strand cDNA synthesis, strand displacement, or second-strand cDNA synthesis; continue to the next step. After full-length cDNA amplification, a brief pause is permitted, and the amplified product may be stored overnight at 4 °C or −20 °C; prompt purification is recommended. Under normal conditions, the purified second-strand cDNA product concentration is 0.7–2 ng/µL. A significant deviation from this range may lead to abnormal amplification yield or product length distribution and ultimately affect sequencing and data performance. Stop point: A quality-control-qualified amplified product can be used directly in downstream procedures, stored short term at −20 °C, or stored long term at −80 °C. Handling Before use, thaw all kit components at room temperature, mix thoroughly by inverting several times, centrifuge briefly, and place on ice. Use RNase-free consumables and clean work areas regularly. Physically separate the area used to prepare PCR reactions from the area used to purify and analyze PCR products. Use dedicated pipettes for library construction and clean each work area regularly to maintain a clean experimental environment. Warning For research use only. For your

	safety and health, wear a lab coat and disposable gloves during operation.
Storage	Storage Store at −25 to −15 °C. Stability Shelf life: 1 year.
Reaction conditions	Preparation Dissolve purified total RNA in nuclease-free H_2O whenever possible. Before starting the reactions, perform RNA quality control to assess RNA integrity, purity, and concentration. Using degraded RNA or RNA with seriously substandard purity as the starting template may result in low cDNA yield, shorter fragments, a substantially reduced proportion of full-length cDNA, and possible reaction failure. Assay Protocol 1. RNA denaturation 1.1 Prepare the RNA denaturation reaction with total RNA x µL (150 ng), Oligo (dT) Primer 1 µL, and nuclease-free H_2O 6 − x µL; total volume: 7 µL. 1.2 Flick the tube to mix the reaction solution and centrifuge briefly to collect the liquid at the bottom of the tube. Place the PCR tube in a thermal cycler and run the RNA-denaturation program: heated lid 85 °C, On; 72 °C for 5 min. Immediately place the tube on ice for at least 3 min. 2. Reverse transcription and strand displacement 2.1 Prepare the reverse-transcription reaction by combining the 7 µL RNA-denaturation reaction, 4 µL 5× RT Buffer, 2 µL 1st Strand Enzyme Master Mix, and 6 µL nuclease-free H_2O; total volume: 19 µL. 2.2 Flick the tube to mix and centrifuge briefly. Run the first-strand cDNA program: heated lid 105 °C, On; 42 °C for 60 min; 20 °C, Hold. Do not pause at this step; continue the procedure. 2.3 After the first-strand reaction, remove the PCR tube and rapidly bring the tube cap to room temperature, for example by contacting the cap with the bottom of an ice box. Add 1 µL TSO Primer to the 19 µL first-strand reaction; total volume: 20 µL. 2.4 Flick the tube to mix and centrifuge briefly. Run the strand-displacement program: heated lid 105 °C, On; 42 °C for 15 min; 70 °C for 15 min; 4 °C, Hold. Do not pause at this step; continue the procedure. 3. Second-strand cDNA synthesis and purification Before starting, equilibrate the magnetic beads at room temperature for at least 30 min and mix thoroughly before use. Prepare 80% ethanol fresh from 100% ethanol and nuclease-free H_2O. 3.1 After strand

displacement, remove the PCR tube and rapidly bring the tube cap to room temperature. Prepare the second-strand cDNA synthesis reaction by combining the 20 µL strand-displacement reaction, 7 µL 2nd Reaction Buffer, 3 µL 2nd Strand Enzyme Mix, and 5 µL nuclease-free H_2O; total volume: 35 µL. 3.2 Flick the tube to mix and centrifuge briefly. Run the second-strand cDNA synthesis program: heated lid Off; 16 °C for 5 min; 4 °C, Hold. Do not pause at this step; continue the procedure. 3.3 Add 0.9× magnetic beads (31.5 µL) to the 35 µL second-strand cDNA synthesis product. Rotate slowly on a rotating mixer at room temperature for 10 min, or incubate at room temperature and flick the tube every 3 min. 3.4 Remove the PCR tube, centrifuge briefly, and place it on a magnetic rack. Wait 2–5 min until the liquid is clear, then carefully remove and discard the supernatant with a pipette. 3.5 While the tube remains on the magnetic rack, add 200 µL freshly prepared 80% ethanol. Incubate for 30 sec and carefully remove and discard the supernatant. 3.6 Repeat the ethanol wash. 3.7 Remove the PCR tube from the magnetic rack, centrifuge briefly, return it to the magnetic rack, and use a small-volume pipette to remove and discard any remaining liquid at the bottom of the tube. 3.8 Keep the PCR tube on the magnetic rack, open the cap, and dry at room temperature until the bead surface is no longer reflective and has no cracks. 3.9 Remove the low-retention tube from the magnetic rack, add 23 µL nuclease-free H_2O for second-strand cDNA elution, and flick the tube until mixed. Centrifuge briefly to collect the liquid at the bottom of the tube. Incubate on a 25 °C heat block for 10 min, flicking every 3 min. 3.10 Centrifuge the PCR tube briefly, place it on a magnetic rack, and wait 2–5 min until the liquid is clear. Transfer 20 µL of the supernatant to a new PCR tube. The remaining sample may be used for concentration measurement. 4. Full-length cDNA amplification and purification Before starting, equilibrate the magnetic beads at room temperature for at least 30 min and mix thoroughly before use. Prepare 80% ethanol fresh from 100% ethanol and nuclease-free H_2O. 4.1 Prepare the cDNA amplification reaction by combining 20 µL purified second-strand cDNA product, 2.5 µL PCR-F, 2.5 µL PCR-R, and 25 µL 2× Multiplex Long PCR Master Mix; total

	volume: 50 µL. 4.2 Flick the tube to mix and centrifuge briefly. Run the full-length cDNA amplification program: heated lid 105 °C, On; 98 °C for 30 sec, 1 cycle; 15 cycles of 98 °C for 10 sec, 60 °C for 5 sec, and 72 °C for 2 min; 72 °C for 2 min, 1 cycle; 4 °C, Hold. A brief pause is permitted at this step. The amplified product may be stored overnight at 4 °C or −20 °C; prompt purification after amplification is recommended. If the total RNA input is below 150 ng, the amplification yield will be low. For example, when UHRR input is reduced to 50 ng, the amplification yield is approximately 1 µg. Increasing the PCR cycle number to obtain sufficient cDNA may introduce additional nonspecific amplification and reduce the proportion of full-length cDNA. 4.3 Add 0.9× magnetic beads (45 µL) to the 50 µL cDNA amplification product. Rotate slowly on a rotating mixer at room temperature for 10 min, or incubate at room temperature and flick the tube every 3 min. 4.4 Remove the PCR tube, centrifuge briefly, and place it on a magnetic rack. Wait 2–5 min until the liquid is clear, then carefully remove and discard the supernatant. 4.5 While the tube remains on the magnetic rack, add 200 µL freshly prepared 80% ethanol. Incubate for 30 sec and carefully remove and discard the supernatant. 4.6 Repeat the ethanol wash. 4.7 Remove the PCR tube from the magnetic rack, centrifuge briefly, return it to the magnetic rack, and use a small-volume pipette to remove and discard any remaining liquid at the bottom of the tube. 4.8 Keep the PCR tube on the magnetic rack, open the cap, and dry at room temperature until the bead surface is no longer reflective and has no cracks. 4.9 Remove the low-retention tube from the magnetic rack, add 35 µL nuclease-free H_2O for DNA elution, and flick the tube until mixed. Centrifuge briefly to collect the liquid at the bottom of the tube. Incubate on a 25 °C heat block for 10 min, flicking every 3 min. 4.10 Centrifuge the PCR tube briefly, place it on a magnetic rack, and wait 2–5 min until the liquid is clear. Transfer 32 µL of the supernatant to a new 1.5 mL low-retention tube. The remaining sample may be used for quality control.
Usage	Usage For research use only. Materials Required but Not Supplied Total RNA

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	sample; nuclease-free H_2O; RNase-free consumables; magnetic beads; 100% ethanol; freshly prepared 80% ethanol; PCR tubes; a thermal cycler with a heated lid; ice; a magnetic rack; a centrifuge; low-retention tubes; 1.5 mL low-retention tubes; pipettes, including dedicated pipettes for library construction; a rotating mixer; a 25 °C metal bath; a UV-Vis spectrophotometer; a fluorescence-based nucleic acid quantification instrument; an automated electrophoresis instrument.

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