

# SOLiD™ Total RNA-Seq Kit for Whole Transcriptome Libraries



**Note:** For safety and biohazard guidelines, refer to the “Safety” section in the *SOLiD™ Total RNA-Seq Kit Protocol* (PN 4452437). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Fragment the whole transcriptome RNA

### 1 Fragment the RNA

a. For each RNA sample, assemble a reaction on ice:

Component	Volume
RNA sample and Nuclease-free Water: <ul style="list-style-type: none"> <li>• Poly(A) RNA: 100–500 ng</li> <li>• rRNA-depleted total RNA: 200–500 ng</li> <li>• WT Control RNA: 500 ng</li> </ul>	8 $\mu$ L
10X RNase III Buffer	1 $\mu$ L
RNase III	1 $\mu$ L
<b>Total volume</b>	<b>10 <math>\mu</math>L</b>

b. Incubate the reaction in a thermal cycler at 37 °C for 10 minutes.

c. **Immediately** after the incubation, add 90  $\mu$ L of Nuclease-free Water, then place the fragmented RNA on ice. Go to the next step immediately, or leave on ice for less than 1 hour.

### 2 Clean up the RNA

Use the RiboMinus™ Concentration Module (Invitrogen).

a. Prepare the Wash Buffer (W5) with ethanol, then store at room temperature:

Component	Volume
100% ethanol	6 mL
Wash Buffer (W5)	1.5 mL

b. Add to the fragmented RNA, then mix well:

- 100  $\mu$ L of Binding Buffer (L3)
- 250  $\mu$ L of 100% ethanol

c. Clean up the RNA:

Step	Load	Spin	Flowthrough
Bind the RNA to the Spin Column	450 $\mu$ L of RNA sample containing Binding Buffer (L3) and ethanol	12,000 $\times$ g for 1 min	Discard
Wash the RNA	500 $\mu$ L of Wash Buffer (W5) with ethanol	12,000 $\times$ g for 1 min	Discard
	–	Max speed for 2 min	Discard
Elute the RNA in a clean Recovery Tube	12 $\mu$ L of RNase-Free Water	Wait 1 min, then max speed for 1 min	Save the RNA

**3 Assess the yield and size distribution of the fragmented RNA**

- a. Quantitate the yield of the fragmented RNA using the Quant-iT™ RNA Assay Kit on the Qubit® Fluorometer (Invitrogen).
- b. Assess the size distribution of the fragmented RNA:
  1. Dilute 1 µL of the sample 1:10 with Nuclease-Free Water.
  2. Run the diluted sample on an Agilent 2100 bioanalyzer with the RNA 6000 Pico Chip Kit.
  3. Use the 2100 expert software to review the size distribution (average size should be 100–200 nt).
- c. Proceed according to the amount of fragmented RNA you have in 3 µL:

Amount of fragmented RNA in 3 µL	Instructions
<ul style="list-style-type: none"> <li>• ≥50 ng poly(A) RNA</li> <li>• ≥100 ng rRNA-depleted total RNA</li> <li>• ≥100 ng WT Control RNA</li> </ul>	Proceed with <a href="#">Construct the amplified whole transcriptome library</a> . Store the remaining RNA at -80 °C.
<ul style="list-style-type: none"> <li>• &lt;50 ng poly(A) RNA</li> <li>• &lt;100 ng rRNA-depleted total RNA</li> </ul>	<ol style="list-style-type: none"> <li>1. Dry 50–100 ng of the RNA completely in a centrifugal vacuum concentrator at low or medium heat (40 °C) for 10–20 minutes.</li> <li>2. Resuspend in 3 µL Nuclease-free Water, then proceed with <a href="#">Construct the amplified whole transcriptome library</a>.</li> </ol>

**Construct the amplified whole transcriptome library**

**1 Hybridize and ligate the RNA**

- a. On ice, prepare a hybridization master mix for all reactions:


Component	Volume for one reaction†
SOLiD™ Adaptor Mix	2 µL
Hybridization Solution	3 µL
<b>Total volume per reaction</b>	<b>5 µL</b>

† Include 5–10% excess volume to compensate for pipetting error.

- b. Transfer 5 µL hybridization master mix to 3 µL of each fragmented RNA sample:
  - 50 ng fragmented poly(A) RNA
  - 100 ng fragmented rRNA-depleted total RNA or fragmented WT Control RNA
- c. Run the 8-µL hybridization reactions in a thermal cycler:

Temperature	Time
65 °C	10 min
16 °C	5 min

- d. Add the RNA ligation reagents to each 8-µL hybridization reaction:
  - 10 µL 2X Ligation Buffer
  - 2 µL Ligation Enzyme Mix

 **IMPORTANT!** If the 2X Ligation Buffer contains a white precipitate, warm it at 37 °C for 2–5 minutes. 2X Ligation Buffer is very viscous; pipet slowly to dispense it accurately.
- e. Incubate the 20-µL ligation reactions in a thermal cycler at 16 °C for 16 hours.

**2** Perform reverse transcription

a. Prepare RT master mix (*without* the ArrayScript™ Reverse Transcriptase) for all reactions:

Component	Volume for each reaction†
Nuclease-free Water	11 µL
10X RT Buffer	4 µL
dNTP Mix	2 µL
SOLiD™ RT Primer	2 µL
<b>Total volume per reaction</b>	<b>19 µL</b>

† Include 5–10% excess volume to compensate for pipetting error.

b. Incubate the RT master mix with the ligated RNA sample:

1. Transfer 19 µL of RT master mix to each 20-µL ligation reaction. Pipet up and down a few times to mix, then spin briefly.
2. Incubate in a thermal cycler with a heated lid at 70 °C for 5 minutes, then snap-cool on ice.

c. Perform reverse transcription:

1. Add 1 µL ArrayScript™ Reverse Transcriptase to each ligated RNA sample. **Gently** vortex to mix thoroughly, then spin briefly.
2. Incubate in a thermal cycler with a heated lid at 42 °C for 30 minutes.



**Note:** The cDNA can be stored at –20 °C for a few weeks, stored at –80 °C for long-term storage, or used immediately.

**3** Purify the cDNA

Use the MinElute® PCR Purification Kit (Qiagen).



**Note:** The kit may be supplied with Buffer PB (without pH Indicator) or Buffer PBI (with pH Indicator). It is not necessary to add pH Indicator to Buffer PB before use.

a. Add Nuclease-free Water and Buffer PB or Buffer PBI to the cDNA:

1. Transfer all of the cDNA (40 µL) to a clean 1.5-mL microcentrifuge tube.
2. Add 60 µL of Nuclease-free Water.
3. Add 500 µL of Buffer PB or Buffer PBI, then mix well.

b. Purify the cDNA:

Step	Load	Spin	Flowthrough
Load the cDNA onto the MinElute column	600 µL of sample containing Buffer PB or Buffer PBI	13,000 × g for 1 min	Discard
Wash the cDNA	750 µL of Buffer PE	13,000 × g for 1 min	Discard
	–	13,000 × g for 1 min	Discard
Elute the cDNA in a clean microcentrifuge tube	10 µL of Buffer EB	Wait 1 min, then 13,000 × g for 1 min	Save the cDNA

**4** Size select the cDNA

Use Novex® pre-cast gel products, 50 bp DNA Ladder, and SYBR® Gold nucleic acid gel stain (Invitrogen).

- a. Prepare and assemble a Novex® 6% TBE-Urea Gel in an XCell SureLock™ Mini-Cell, using 1X TBE Running Buffer, as described in the *Novex® Pre-Cast Gel Electrophoresis Guide* by Invitrogen.
- b. Prepare the cDNA and the 50 bp DNA ladder, then load the gel:
  1. Dilute the 50 bp DNA Ladder to 40 ng/μL: 1 μL 50 bp DNA Ladder + 24 μL RNase-free water.
  2. Mix 5 μL of the 40 ng/μL 50 bp DNA Ladder with 5 μL of 2X Novex TBE-Urea Sample buffer.
  3. Mix 5 μL of the cDNA with 5 μL of 2X Novex TBE-Urea Sample Buffer.
  4. Heat the samples at 95 °C for 3 minutes.
  5. Snap-cool on ice, then load. Leave on ice for less than 30 minutes before loading.



**Note:** Immediately before you load each sample, flush the well of the gel several times with 1X TBE Running Buffer.

- c. Run the gel at 180 V until the leading dye front is 1 cm below the middle of the gel (~25 minutes).
- d. Add 5 μL of SYBR® Gold nucleic acid gel stain to 50 mL of 1X TBE Running Buffer, then stain the gel for 5–10 minutes.
- e. Illuminate the stained gel, then excise the gel containing 150–250 nt cDNA.
- f. Transfer the gel piece to a clean working area, then cut the gel vertically into 4 pieces using a clean razor blade. Each gel slice should be about 1 mm × 6 mm.



- g. Place the two gel slices from the middle of the lane individually into clean 0.2-mL PCR tubes for the PCR, and place the outside gel slices into a clean 1.5-mL microcentrifuge tube for storage.

**5** Amplify the cDNA

- a. Prepare 98 μL PCR mix for each gel slice:

Component	Volume	
	One 100-μL reaction	Two 100-μL reactions†
Nuclease-free Water	76.8 μL	169.0 μL
10X PCR Buffer	10.0 μL	22.0 μL
dNTP Mix	8.0 μL	17.6 μL
SOLiD™ 5′ PCR Primer	2.0 μL	4.4 μL
AmpliTaq® DNA Polymerase	1.2 μL	2.6 μL
<b>Total volume</b>	<b>98.0 μL</b>	<b>215.6 μL</b>

† Includes 10% excess volume to compensate for pipetting error.

- b. Transfer 98 μL PCR mix into each 0.2-mL PCR tube with a gel slice.
- c. Add 2 μL SOLiD 3′ PCR Primer to each tube.



**Note:** For multiplex SOLiD System sequencing, substitute with barcoded SOLiD™ 3′ PCR Primers from a SOLiD™ RNA Barcoding Kit.

5 Amplify the cDNA (continued)

d. Run the PCR reactions in a thermal cycler:

Stage	Temp	Time
Hold	95 °C	5 min
Cycle (15 cycles)	95 °C	30 sec
	62 °C	30 sec
	72 °C	30 sec
Hold	72 °C	7 min

6 Purify the amplified DNA

Use the PureLink™ PCR Micro Kit (Invitrogen).

a. Prepare the sample:

1. Combine the two 100-µL PCR reactions in a new 1.5-mL tube.



**IMPORTANT!** If you used two different barcoded SOLiD 3' Primers for your sample, do not combine the PCRs at this step.

2. Add 800 µL of Binding Buffer (B2) to the tube, then mix well.

b. Purify the amplified DNA:

Step	Load	Spin	Flowthrough
Load the sample onto the PureLink™ Micro Kit Column	500 µL of sample containing Binding Buffer (B2)	10,000 × g for 1 min	Discard
Load the remaining half of the sample onto the PureLink™ Micro Kit Column	500 µL of sample containing Binding Buffer (B2)	10,000 × g for 1 min	Discard
Wash the DNA	600 µL of Wash Buffer (W1)	10,000 × g for 1 min	Discard
	–	14,000 × g for 1 min	Discard
Elute the DNA into a clean PureLink™ Elution Tube (1)	10 µL of Elution Buffer	Wait 1 min, then 14,000 × g for 1 min	Save the DNA
Elute the DNA into a clean PureLink™ Elution Tube (2)	10 µL of Elution Buffer	Wait 1 min, then 14,000 × g for 1 min	Save the DNA

7 Assess the yield and size distribution of the amplified DNA

a. Measure the concentration of the purified DNA with a NanoDrop® spectrophotometer, and if necessary, dilute the DNA to <50 ng/µL for accurate quantitation with the DNA 1000 Kit.

b. Run 1 µL of the purified DNA on an Agilent 2100 Bioanalyzer with the DNA 1000 Kit (Agilent).

c. Use the 2100 expert software to assess the yield and size distribution:

1. Perform a smear analysis to quantify the percentage of DNA that is 25–200 bp.

Proceed with SOLiD™ System templated bead preparation only if <20% of the amplified DNA is in the 25–200-bp size range.

2. Determine the median peak size (bp) and molar concentration (nM) of the cDNA library.

8 Proceed with SOLiD System templated bead preparation

Emulsion PCR library concentrations (singleplex or multiplex sequencing pools) of 0.4 pM and 0.8 pM for ePCR are recommended for workflow analysis. Refer to the *Applied Biosystems SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378).

## Materials

For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Item	Source
SOLiD™ Total RNA-Seq Kit	Applied Biosystems PN 4445374
SOLiD RNA Barcoding Kits (optional) For a complete list of available kits, go to <a href="http://www.appliedbiosystems.com">www.appliedbiosystems.com</a> .	Applied Biosystems
8-strip PCR Tubes & Caps, RNase-free, 0.2-mL	Applied Biosystems PN AM12230
Non-Stick RNase-free Microfuge Tubes (0.5 mL), 500	Applied Biosystems PN AM12350
Non-Stick RNase-free Microfuge Tubes (1.5 mL), 250	Applied Biosystems PN AM12450
Nuclease-free Water (not DEPC-treated), 100 mL	Applied Biosystems PN AM9938
50 bp DNA Ladder	Invitrogen PN 10416-014
Novex® 6% TBE-Urea Gels 1.0 mm, 10 well	Invitrogen PN EC6865BOX
Novex® TBE Running Buffer (5X), 1 L	Invitrogen PN LC6675
Novex® TBE-Urea Sample Buffer (2X), 10 mL	Invitrogen PN LC6876
PureLink™ PCR Micro Kit, 50 preps	Invitrogen PN K310050
Quanti-iT™ RNA Assay Kit, 100 assays	Invitrogen PN Q32852
RiboMinus™ Concentration Module, 6 preps  <b>Note:</b> The RiboMinus™ Concentration Module is not equivalent to the RiboMinus™ Eukaryote Kit for RNA-Seq or to the RiboMinus™ Plant Kit for RNA-Seq.	Invitrogen PN K1550-05
SYBR® Gold nucleic acid gel stain, 10,000X concentrate in DMSO, 500 µL	Invitrogen PN S-11494
Agilent DNA 1000 Kit	Agilent PN 5067-1504
Ethanol, 100%, ACS reagent grade or equivalent	MLS
MinElute® PCR Purification Kit (50)	Qiagen PN 28004
Pipette tips, RNase-free	MLS
RNA 6000 Pico Kit	Agilent PN 5067-1513

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