CELEMICS

Kit User Manual

cDNA Synthesis Kit

Version 1.0

Research Use Only

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Materials Provided

Table 1. cDNA Synthesis Kit

Storage condition: -20°C

Box No.	Product Name	1 rxn (μL)	Cat No. CS1016 (16 rxn)	Cat No. CS1024 (24 rxn)	Cat No. CS1048 (48 rxn)	Cat No. CS1096 (96 rxn)
CS10	5X RNA Fragmentation Buffer	2	CS100116	CS100124	CS100148	CS100196
	Primer	1	CS100216	CS100224	CS100248	CS100296
	dNTP Mixture	1	CS100316	CS100324	CS100348	CS100396
	1st Strand Synthesis Buffer 1	4	CS100416	CS100424	CS100448	CS100496
	1st Strand Synthesis Buffer 2	1	CS100516	CS100524	CS100548	CS100596
	RNase Inhibitor	1	CS100616	CS100624	CS100648	CS100696
	Reverse Transcriptase	1	CS100716	CS100724	CS100748	CS100796
	2nd Strand Synthesis 10X Buffer 1	5	CS100816	CS100824	CS100848	CS100896
	2nd Strand Synthesis Enzyme 1	1	CS100916	CS100924	CS100948	CS100996
	2nd Strand Synthesis Enzyme 2	4.5	CS101016	CS101024	CS101048	CS101096
	2nd Strand Synthesis Buffer 2	1.5	CS101116	CS101124	CS101148	CS101196
	2nd Strand Synthesis Enzyme 3	1	CS101216	CS101224	CS101248	CS101296

Additional Purchasable Materials

Table 2. Additional Purchasable Materials List

Product Name	Cat No.	Storage Condition (°C)	Comment
Celemics DNA purification bead (CeleMag Clean-up Bead)	CMCB50 (50 mL) CMCN500 (500 mL)	4°C	Included in All-in-one kit
Celemics Streptavidin bead (CeleMag Streptavidin Bead)	CMSB02 (2 mL) CMSB10 (10 mL)	4°C	
CLM Polymerase Amplification Kit PCR Ready Mix only	CMPFX.XX (X.XX: amount, mL)	-20°C	

Materials Required (Not Provided)

Table 3. Not Provided Materials List

Product Name	Suggested Supplier	
Reagents & Consumables		
Nuclease-free water (not DEPC-treated)	General lab supplier	-
Universal Human RNA	General lab supplier	-
Ethanol (200 proof)	General lab supplier	-
Conical centrifuge tubes (15 mL or 50 mL)	General lab supplier	-
P10,P20,P200 and P1000 pipettes	General lab supplier	-
Multichannel pipette	General lab supplier	-
Sterile, nuclease–free aerosol barrier pipette tips	General lab supplier	-
0.2 mL PCR Tube Strips, Low Profile and Cap Strips	General lab supplier	-
Powder-free gloves	General lab supplier	-
DNA LoBind Tubes, 1.5 mL PCR clean, 250 pieces	Eppendorf	p/n: 022431021 or equivalent
Microtubes 1.5ml Clear, Sterile	AXYGEN	p/n: MCT-150-C-S or equivalent
Eppendorf™ twin.tec™ 96 Well LoBind PCR Plates, Skirted	Eppendorf	p/n: 0030129512 or equivalent
Microseal 'B' adhesive seals	Bio-Rad	p/n: MSB-1001 or equivalent
Equipment		
TapeStation Instrument	Agilent Technologies, Inc.	p/n: G2991BA or equivalent
Magnetic separator	Invitrogen	p/n: 12321D or equivalent
Thermal Cycler	General lab supplier	-
Micro-centrifuge	General lab supplier	-
Vortex mixer	General lab supplier	-
Timer	General lab supplier	-

Before you begin

It is recommended to read the manual carefully to understand the experimental procedure. In particular, read and follow the text **highlighted in bold**.

Notice

The results of this experiment can be affected by the quality of the DNA. DNA extracted from FFPE tissue is highly variable in quality due to chemical damage and fragmentation, and often is available only in small amounts. There is a possibility that these steps will not lead to success with DNA extracted from FFPE samples. Therefore, it is recommended to use high-quality DNA samples.

Directions for the use of this product

1. This product should be used for research use only.
2. Handle specimens cautiously to avoid potential infections.
    1) Handle reagents cautiously and avoid contact with any area of the user's skin, eyes, and mucous membrane. In the event of reagent contact, rinse with water immediately to remove. If the reagent spills from the cartridge, dilute with water and follow the duly accredited treatment.
    2) All blood serum materials from human sources should be handled carefully and treated as potential infectious sources.
3. Genomic DNA/RNA extracted from bio-specimens such as blood, saliva, FFPE, and fresh tissue should be used as sample specimens.
4. Reagents should be used in accordance with the suggested Usage and Volume within the expiry date.
5. Reagent storage should be carried out at their suggested temperature and condition. Temperature conditions are listed below:

    Room temperature (RT, 15-25˚C), Freezer(-20˚C), Deep freezer (-80˚C)
6. Consult an authorized management company for proper management of used and expired reagents in accordance with the relevant laws.
7. Be cautious with specific allergenic reagents.

Please maintain a nuclease-free environment during the experiment. Wear a lab coat, disposable gloves and protective goggles. If you have any problems, questions, or related concerns, please email us at support@celemics.com

Protocol Overview

NGS Library Preparation workflow	Time
RNA Fragmentation	0.5 hour
1st & 2nd Strand Synthesis, Purification using CeleMag Clean-up Beads	2.5 hours

Sample Preparation

Step 1. RNA Fragmentation 8

Step 2. 1st Strand Synthesis 10

Step 3-1. 2nd Strand Synthesis-1 12

Step 3-2. 2nd Strand Synthesis-2 13

Step 4. 2nd Strand Synthesis Clean-up..14

Step 1. RNA Fragmentation

[Note]

To minimize 3'→5' bias, the RNA is fragmented at high temperature in the presence of magnesium. RNA will be fragmented to the desired size by incubation at high temperature.

[Preparation]

- cDNA Synthesis Kit → 5X RNA Fragmentation Buffer
- RNase-free water

[Procedure]

1. Prepare **5X RNA Fragmentation Buffer Mix** (Table 4) in a new PCR tube, mix by pipetting and pulse-spin down the tube.

   * Important – Do not vortex RNA sample and mixture including RNA.

   Table 4. 5X RNA Fragmentation Buffer Mix

Reagent	1 Reaction (μL)
5X RNA Fragmentation Buffer	2
RNA sample (25 ng – 1 μg)	X
RNase-free water	(8 - X)
Total volume	10.0

2. Put the tube in the thermal cycler and run the program as shown in Table 5.

   Table 5. Recommended fragmentation conditions (Lid Temperature: 100°C)

Input RNA quality	DV_{200}	Fragmentation
High	> 70%	6 - 7 min. at 94°C
Medium	50 - 70%	1 min. at 94°C
Low	30 - 50%	1 - 6 min. at 85°C
Too Degraded	< 30%	30 sec. at 65°C

   * DV_{200} value means the percentage of fragments with > 200 nucleotides.

3. When the fragmentation step has finished, put the tube **on ice** and Proceed with the 1st Strand Synthesis immediately.

If starting with RNA sample amount below 25 ng, use carrier RNA (e.g. Universal Human RNA) and Proceed with steps described below.

1. Prepare 5X RNA Fragmentation Buffer Mix (Table 6) in a new PCR tube, mix by pipetting and pulse-spin down the tube.

   * Important – Do not vortex sample mixture including RNA.

   Table 6. 5X RNA Fragmentation Buffer Mix

Reagent	1 Reaction (μL)
5X RNA Fragmentation Buffer	2
RNA sample (<25 ng)	X
Carrier RNA (100 ng)	Y
RNase-free water	8-(X+Y)
Total volume	10

2. Put the tube in a thermal cycler and incubate at 94°C for 7 minutes.

   * Lid temperature: 100°C

3. When the fragmentation step has finished, put the tube on ice and Proceed with the 1st Strand Synthesis immediately.

Important
Proceed with the next step right away.

Step 2. 1st Strand Synthesis

[Note]

Fragmented RNA is reverse transcribed with random primers to make 1st strand cDNA.

[Preparation]

- cDNA Synthesis Kit → 1st Strand Synthesis Buffer 1, 1st Strand Synthesis Buffer 2, Reverse Transcriptase, dNTP mixture, Primer, RNase Inhibitor
- RNase-free water

[Procedure]

1. Prepare **1st Strand Synthesis Primer Mix** to anneal random primer to template RNA in a reaction tube as shown in Table 7.

   * Important – Combine all reagents on ice.

   Table 7. 1st Strand Synthesis Primer Mix

Reagent	1 Reaction (μL)
Fragmented RNA	10
Primer	1
dNTP mixture	1
RNase-free water	1
Total volume	13

2. Mix well by pipetting and pulse-spin down. Put the tube in the thermal cycler and run the program as shown in Table 8.

   Table 8. 1st Strand Synthesis Primer Mix

Step	Temperature	Time
Primer Annealing	65°C	5 min.
Cooling	ice	At least 1 min.

3. Prepare **1st Strand Synthesis Master Mix** as shown in Table 9.

   Table 9. 1st Strand Synthesis Master Mix

Reagent	1 Reaction (μL)
Random hexamer annealed RNA	13
1st Strand Synthesis Buffer 1	4
1st Strand Synthesis Buffer 2	1
RNase Inhibitor	1
Reverse Transcriptase	1
Total volume	20

4. Mix well by pipetting and pulse-spin down. Put the tube in the thermal cycler and run the program as shown in Table 10.

Table 10. 1st Strand Synthesis Program

Step	Temperature	Time
Primer extension	23°C	10 min.
1st Strand Synthesis	50°C	10 min.
Enzyme inactivation	80°C	10 min.
HOLD	4°C	∞

5. When the 1st Strand Synthesis step has finished, put the tube **on ice** and Proceed with 2nd Strand Synthesis-1 immediately.

Important

Proceed with the next step right away.

Step 3-1. 2nd Strand Synthesis-1

[Note]

This step converts cDNA:RNA hybrid to dscDNA (double stranded cDNA).

[Preparation]

- cDNA Synthesis Kit → 2nd Strand Synthesis Enzyme 1,
  2nd Strand Synthesis Enzyme 2,
  2nd Strand Synthesis 10X Buffer 1
- RNase-free water

[Procedure]

1. Prepare **2nd Strand Synthesis-1 Mix** as shown in Table 11 and mix the tube well.

   Table 11. 2nd Strand Synthesis-1 Mix

Reagent	1 Reaction (μL)
1st Strand Synthesis product	20
2nd Strand Synthesis 10X Buffer 1	5
2nd Strand Synthesis Enzyme 1	1
2nd Strand Synthesis Enzyme 2	4.5
RNase-free water	19.5
Total volume	50

2. Pulse-spin down and put the tube in the thermal cycler and run the program as shown in Table 12.

   Table 12. DNase Digestion Master Mix

Step	Temperature	Time
2nd Strand Synthesis-1	16°C	60 min.
HOLD	4°C	∞

3. When the 2nd Strand Synthesis-1 step has finished, put the tube **on ice** and Proceed with 2nd Strand Synthesis-2 immediately.

Important

Proceed with the next step right away.

Step 3-2. 2nd Strand Synthesis-2

[Preparation]

- cDNA Synthesis Kit → 2nd Strand Synthesis Buffer 2,
  2nd Strand Synthesis Enzyme 3

[Procedure]

1. Prepare **2nd Strand Synthesis-2 Mix** as shown in Table 13 and mix the tube well.

Table 13. 2nd Strand Synthesis-2 Mix

Reagent	1 Reaction (µL)
2nd Strand Synthesis-1 product	50
2nd Strand Synthesis Buffer 2	1.5
2nd Strand Synthesis Enzyme 3	1
Total volume	52.5

2. Pulse-spin down and put the tube in the thermal cycler and run the program as shown in Table 14.

Table 14. Thermal cycler program (2nd Strand Synthesis-2)

Step	Temperature	Time
2nd Strand Synthesis-2	25°C	15 min.
HOLD	4°C	∞

3. When the 2nd Strand Synthesis-2 step has finished and block temperature reaches 4°C, put the tubes on ice and Proceed with 2nd Strand Synthesis Clean-up immediately.

Important

Proceed with the next step right away.

Step 4. 2nd Strand Synthesis Clean-up

[Preparation]

- CeleMag Clean-up Beads
  Keep the beads at room temperature for at least 30 minutes before use.
  Store CeleMag Clean-up Beads at 4°C after use.
- 80% ethanol solution
- Nuclease-free water

[Procedure]

1. Thoroughly vortex CeleMag Clean-up Beads in order to achieve a homogeneous state.
2. **Add 94.5 µL CeleMag Clean-up Beads** to a new 1.5 ml LoBind Tube and **add 2nd Strand Synthesis and Marking sample** to the tube. Combine well on a vortex mixer and pulse-spin down the tube. Incubate at room temperature for 5 minutes (*avoid spinning down the CeleMag Clean-Up Beads too strongly to prevent precipitation*).

   Table 15. Volumes of CeleMag Clean-up Beads (1.8X)

Reagent	Volume Ratio (Beads : Sample)
CeleMag Clean-up Beads	**1.8 : 1** 94.5 µL : 52.5 µL

3. Put the tube in a magnetic separator for **3-5 minutes** until the solution is clear.
4. Keep the tube in the magnetic separator and discard the supernatant *(be careful not to touch the beads while you remove the supernatant).*
5. Keeping the tube in the magnetic separator, **add 500 µL (180 µL for PCR tube or 96 well plate) fresh 80% ethanol** to each sample.
6. Incubate for 30 seconds at room temperature, then discard the ethanol *(be careful not to touch the beads while you remove the supernatant).*
7. Repeat the 80% ethanol wash (**step 5-6**).
8. Spin down and put the tube in magnetic separator and discard residual ethanol.
9. **Dry the sample tube** at room temperature for **2 minutes** or **until residual ethanol has completely evaporated** (*avoid drying beads until pellets appear cracked, as excessive drying decreases elution efficiency*).
10. **Add 37 µL of nuclease-free water** to the sample and mix well on a vortexer. Pulse-spin down and incubate the sample at room temperature for **2 minutes**.
11. Put the tube in the magnetic separator for **2 minutes** until the solution is clear.
12. **Transfer the supernatant (35 µL)** to a new 1.5mL LoBind tube.

Stop Point

If not continuing to the next step, the sample can be stored at -20°C.