§§ Charm Biotech

Just-a-Tube™ Laser Captured Microdissection (LCM) Sample Total RNA/MicroRNA Purification Kit

Store at room temperature, store Proteinase K at 4 °C

Product Contents and Storage

The components included with **Just-a-Tube** LCM sample RNA and MicroRNA purification kit are listed below. Upon receipt store all components at room temperature except Proteinase K, which needs to be stored at 4 °C. Sufficient reagents are included to perform 25, 50 or 250 LCM nucleic acid isolations.

Product Cat. #	CL-150-M	CL-150-L	CL-150-S
Purification Scale	50	250	25
Binding Tube (BT5)	50	250	25
Digestion Buffer RD1 (RD1)	3.0 ml	15.0 ml	1.5 ml
Digestion Buffer RD2 (RD2)	3.0 ml	15.0 ml	1.5 ml
Binding Buffer (RB7)	1.8 ml	9.0 ml	0.9 ml
Washing Buffer II (WB2)	2.5 ml	12.5 ml	1.25 ml
Elution Buffer (EB3)	1.8 ml	9.0 ml	0.9 ml
Proteinase K (PK2) (20 mg/ml)	0.4 ml	2.0 ml	0.2 ml

Product Description

The Just-a-Tube Laser Captured Microdissection (LCM) Sample Total RNA and MicroRNA Purification Kit is designed and optimized for easy and fast purification of RNA directly from frozen and FFPE cells and tissues with maximum recovery efficiency. No spin-column, filter plate, silica membrane, or magnetic beads are needed for the clean-up and no vacuum or filtration steps are required in the process. Based on Charm Biotech developed Solid Surface Reversible Binding (SSRB) technology, the Just-a-Tube system utilizes micro-tube coated with proprietary turbo-binders acting to selectively capture and efficiently bind RNA from LCM cell lysates. In the presence of Binding Buffer RNA specifically interacts with the turbo binders and binds to the tubes while proteins and other contaminants will remain in the solution. Unbound material is removed in washing steps. The purified RNA can easily be eluted in 10 mM Tris Elution Buffer or water, or at your option, to perform downstream applications such as RT-PCR, qRT-PCR and other gene expression analysis directly in the same tube without sample elution. RNA molecules from as few as several cells can be efficiently isolated and detected with high-quality consistently. Since RNA purification and RNA analysis all can be processed in the same tube without sample transferring between tubes or columns as in other purification methods, opportunities for cross-contaminations are greatly minimized, and the sample recovery efficiency is greatly maximized comparing silica membrane or magnetic beads-based methods. This feature is extremely important for precious samples with limited resources such as LCM samples.

Feature Highlights

Easy to handle: All procedures have been optimized with single tube for ease-of-use. You can perform RNA purification and downstream assays all in the same tube without sample transferring.

All type RNA preservation: Current protocol is engineered for isolation of total RNA including microRNA without tedious multiple spinning procedure used in the column-based method. Low, medium, and high-abundant genes are consistently isolated and preserved in the purified sample ready for gene expression analysis.

Maximum recovery: The biggest issue centering around LCM sample nucleic acid isolation is extremely limited starting materials. Capable of maximizing nucleic acid recovery from LCM samples is the unique feature of the kit. Unlike conventional silica membrane or bead-based purification platform, there are no void spaces in the Binding Tube. This novel solid-surface capturing technology prevents washing buffer carry-over and sample trapping problems in their void space associated with silica membrane or bead-based purification methods.

This feature not only allows fast buffer washing and easy sample elution, but also provides maximum sample capture and release from limited LCM starting samples.

Additional Materials Needed

- 100 % ethanol and Isopropanol (ACS grade or better)
- β-mercaptoethanol (2-Mercaptoethanol) (β-ME) (@14.3 M) or DTT (@1.0 M)
- DNase I and its buffer
- Single-channel pipettor and RNase-free tips
- Micro-centrifuge and spin adaptor for 0.2-0.5 ml micro-tubes, such as Eppendorf Centrifuge and spin adaptor
- MMI Capture Tube or Arcturus Capture HS Cap and matched tube for the HS Cap or PALM Laser System collection cap/tube
- Incubator and/or Thermocycler

General Precautions

- This kit is for research use only. All due care and attention should be exercised in the handling of the kits.
- Wear a laboratory coat, disposable gloves, and eye protection when handling reagents and tubes. Avoid ingestion and inhalation of reagents. In case of contact, wash thoroughly with water. See Material Safety Data Sheets (MSDS) for emergency procedures in case of accidental contact or ingestion. MSDS information is available upon request.
- Always use proper aseptic techniques to avoid nuclease contamination when working with RNA. Use only sterile, new pipette tips to prevent cross contamination.

Preparation Before Starting

- For CL-150-S kit, add 5 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. For CL-150-M kit, add 10 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. For CL-150-L kit, add 50 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. Mark bottle that ethanol has been added! – Store at room temperature and use WB2 containing ethanol within six (6) months.
- Prepare fresh working Digestion Buffer RD1 (RD1) if you use LCM samples from frozen tissue sections, or fresh working Digestion Buffer RD2 (RD2) if you use LCM samples from FFPE tissue sections.

  Add 9 µl of Proteinase K (provided) and 1 µl of 14.3 M β-mercaptoethanol (not provided) or 1.0 M DTT (not provided) to each 90 µl of Digestion Buffer (RD1 or RD2) to reach final 1% (v/v) β-mercaptoethanol or 10 mM DTT concentration and mix well. Add 50 µl working Digestion Buffer for each LCM sample.
- Prepare fresh working Binding Buffer (RB7) prior to performing RNA isolation procedure based on the number of samples processed. To make fresh working Binding Buffer (RB7), for each 10 µl of Binding Buffer (RB7) add 40 µl of 100% isopropanol. Mix well. Prepare a master working Binding Buffer solution based on (1) the number of samples processed and (2) any anticipated loss, generally 10 %, during dispensing. Dispense 100 µl of Binding Buffer containing isopropanol per 50-µl final cell lysates. Discard the unused Binding Buffer at the end of the day.
- The kits are designed to purify total RNA and microRNA from as few as one cell up to 100000 cells. Please following LCM procedure recommended by manufactures exactly to minimize sample degradation before proceeding RNA purification listed in the protocol.
- If desired, you may add an internal control into the purification procedure. Internal control RNA should be added together with the binding buffer.

Experimental Procedure

Digesting LCM Samples

1. After performing LCM capture of cells or tissues, if you use MMI LCM method, add 50 µl Digestion Buffer to the capturing tube and close the tube cap. If you use Arturus LCM method or PALM Microlaser System, add 50 µl Digestion Buffer to a 0.2 ml or 0.5 ml microtube (not provided) which is matched with original capturing HS Cap or collection cap. Close the cap tight. Invert the tube and tap the cap with a finger until the solution spreads and covers the whole cap completely.

   For LCM samples from frozen tissues or cells: Use working Digestion Buffer RD1 (RD1) premixed with Proteinase K and β-mercaptoethanol or DTT.

   For LCM samples from FFPE tissues: Use working Digestion Buffer RD2 (RD2) premixed with Proteinase K and β-mercaptoethanol or DTT.
2. Incubate the inverted tube at 52 °C for frozen tissues or at 60 °C for FFPE tissues for 10-15 minutes to allow the captured cells and tissues detaching from the cap into the digestion buffer.
3. Centrifuge briefly to collect all liquid at the bottom of the tube. Mix the liquid by pipetting up and down the solution several times to disperse any pellet precipitate.
4. Incubate the tube at 52°C or 60 °C in a thermocycler or incubator for complete cell/tissue digestion.

   For LCM samples from frozen tissues or cells: Incubate at 52°C for 1-2 hour with occasional mixing until lysis is complete.

   For LCM samples from FFPE tissues: Incubate at 60 °C for 3-4 hour with occasional mixing until lysis is complete.

(Optional: For FFPE tissue samples, you may perform digestion at 48 °C overnight if preferred).

At the end of incubation, no pellets should be visible, otherwise, extend incubation 15 minutes more to make sure no pellets exist. (Note: When using a thermocycler for incubation, please use thermocycler's hot-lid function to prevent evaporation.).

5 Centrifuge briefly to collect all liquid at the bottom of the tube.

Binding RNA Products

1. Transfer all 50 µl cell lysate solution from the capturing tube to a Binding Tube (BT5).
2. Add 100 µl fresh working Binding Buffer (RB7) containing ethanol to the Binding Tube (BT5). Mix well with the lysate by pipetting up and down the solution 10 times to obtain a homogenous solution.
3. Centrifuge the Binding Tube (BT5) at maximum speed (about 13000 rpm or 16000 X g) at room temperature for 8 minutes to bind the RNA. (Note: During centrifugation, always position the micro-tube hinge pointed outward from the center of rotation. Majority of RNA will collect at the bottom along the hinge side of the Binding Tube.)
4. Remove the solution from the tube. Choose one of the methods listed below to remove the solution. (1) Decant the solution by quick flip-over the tube over a waste container and shaking briskly, then put the inverted tube on a stack of clean absorbent paper such as Kimwipe, or paper towels, then tap the tube on the clean paper to remove as much liquid as possible; or (2) Remove the solution by aspirating the solution from the exact center of the tube bottom with a pipette tip. Be sure not to scrape the walls of the tube with pipette tips during aspiration as the products are bound to the walls of the Binding Tube.

Washing Products

1. Add 200 µl Wash Buffer II (WB2) containing ethanol to the Binding Tube (BT5). Incubate the tube for 30 seconds at room temperature.
2. Centrifuge the Binding Tube (BT5) at maximum speed (about 13000 rpm or 16000 X g) at room temperature for one minute. (Note: During centrifugation, always position micro-tube hinge pointed outward from the center of rotation.)
3. Remove the Wash Buffer II (WB2) from the Binding Tube using one of the methods suggested in step 4 of "Binding RNA Products".
4. Repeat step 1 to 3 above for a total of two washes with Washing Buffer II (WB2).
5. After the final wash, to ensure complete removal of Washing Buffer, spin the Binding Tube (BT5) very briefly, aspirate the last drop of liquid at the bottom of the tube with a 200 µl pipet tip, and air-dry the Binding Tube (BT5) in a lab hood for 8 – 10 minutes to remove any residual liquid.

DNase I Digestion Eluting RNA Products

Sample Elution

RNA attached to the walls of the Binding Tube can be stored directly in the tube at –80 °C for long-term storage until further use. If the RNA is to be eluted for analysis before DNase I digestion, please follow the procedure below.

1. Add 20 µl Elution Buffer (EB3) into each tube.
2. Close the tube and vortex the tube for 10 - 30 seconds, or tap the Binding Tube (RT5) 10 - 15 times with a finger.
3. Briefly centrifuge the tube to collect all solution, and place the tube on ice for downstream applications. If you are not going to analyze the samples immediately, store the tube at -80 °C until use.

If DNase I digestion procedure is needed, please directly proceed to the DNase I digestion procedure without sample elution first.

DNase I Digestion

To prevent any gDNA contamination, DNase I (not provided) digestion is required for removal of gDNA. When choosing DNase I, please make sure to use RNase-free DNase I.

1. DNase I digestions can be performed directly in the Binding Tube. For DNase I digestion, use a final reaction volume of 20 µl for each sample. Please follow the DNase I supplier's instructions for DNase I digestion and inactivation directly in the Binding Tube. (Note: After inactivation of DNase, RNA sample can be used directly for downstream applications.)
2. Once the DNase I digestion and inactivation are completed, centrifuge the Binding Tube briefly and place it on ice for further downstream applications. If you are not going to analyze the samples immediately, store the tube at -80 °C until use.

Electrophoresis and Downstream Application

The size of purified RNA can be examined by gel electrophoresis. Quantity of purified RNA may be determined by UV absorbance at 260 nm or fluorescent RNA assay. Purified RNA in the tube can be used immediately for downstream gene expression analysis.

Troubleshooting

Problem	Cause	Solution
Low yield of product	Number of cells used was less than recommended or Poor quality of starting material	Be sure the protocol for LCM cell capture, collection and processing is followed correctly. Increase the number of captured cells if possible.

	Incomplete lysis	If incomplete lysis is observed, extend the Proteinase K digestion time for an addition of 15 – 30 minutes.
	Low centrifugation forces	Make sure the Binding Tube was spun at ~16000 x g for 10 minutes. If lower speed is used, increase the spin time.
	Pipette tips scrap the wall of the Binding Tube too much during aspiration of solution	Be sure to put the pipette tip at the exact bottom center of the Binding Tube during aspiration.
	RNase contamination in the DNase I	Make sure DNase I is RNase free
No RT-PCR product	Missing Component in the RT-PCR mixture	Be sure to add all components. Check positive control and negative control for PCR reaction.