

Just-a-Tube™ Laser Captured Microdissection (LCM) Sample Genomic DNA 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 gDNA 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 50 or 250 LCM genomic DNA isolations.

Product Cat. #	CA-160-M	CA-160-L
Purification Scale	50	250
Binding Tube (BT3)	50	250
Digestion Buffer 1 (DD1)	6.0 ml	30.0 ml
Digestion Buffer 2 (DD2)	6.0 ml	30.0 ml
Binding Buffer (PB5)	12.0 ml	60.0 ml
Washing Buffer II (WB2)	6.0 ml	30.0 ml
Elution Buffer (EB1)	2.0 ml	10.0 ml
Proteinase K (PK2) (20 mg/ml)	0.6 ml	3.0 ml

Product Description

The Just-a-Tube Laser Captured Microdissection (LCM) Sample Genomic DNA Purification Kit is designed and optimized for easy and fast single-tube purification of gDNA 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-tubes coated with proprietary turbo-binders acting to selectively capture and efficiently bind genomic DNA from LCM cell lysates. In the presence of Binding Buffer gDNA 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 gDNA can easily be eluted in 10 mM Tris Elution Buffer or water, or at your option, to perform downstream applications such as PCR, qPCR and SNP analysis directly in the same tube without sample elution. DNA molecules from as few as several cells can be efficiently isolated and detected with high-quality consistently. Since gDNA purification and DNA 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 bead-based methods. This feature is extremely important for small and precious samples such as LCM samples.

Feature Highlights

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

Reliable quality: Just-a-Tube LCM gDNA purification kit can provide highly pure gDNA with consistent tube-to-tube performance. No PCR inhibitors (such as heme, melanin, hematoxylin or other dyes which are usually carried over from tissues preparation into the extraction solution in other single-tube DNA extraction methods) exist in the final solutions.

Maximum recovery: The biggest issue centering around LCM sample gDNA 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

- 96-100 % ethanol
- DTT for sperm samples
- MMI Capture Tube or Arturus Capture HS Cap and its matched microtube

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. Avoid skin contact with reagents in the kit. In case of contact, wash thoroughly with water. See Material Safety Data Sheets (MSDS) for emergency procedures in case of accidental contact or ingestion. All MSDS information is available upon request.
- Always use proper aseptic techniques to avoid nuclease contamination when working with nucleic acids and use only sterile, new pipette tips to prevent cross contamination.

Preparation Before Starting

- For CA-160-M kit, add 24 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. For CA-160-L kit, add 120 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 1 (DD1) if you use LCM samples from frozen tissue sections and sperm:
Add 10 µl of Proteinase K to each 90 µl of Digestion Buffer 1(DD1) and mix well. To isolate gDNA from sperm, add DTT to 10 mM final concentration in the Digestion Buffer 1 (DD1) mixed with Proteinase K.
(Note: Digestion Buffer 1 (DD1) contains SDS, which can precipitate out of the buffer if stored at room temperature for a long time. If a precipitate is present, incubate the buffer in a 37 °C-58 °C water bath for 5 minutes, or until the SDS re-dissolves and the solution clears. Mix thoroughly but swirling gently.)
- The kits are designed to purify gDNA from as few as several cells up to 100000 cells. Please following LCM procedure recommended by manufacturers exactly to minimize sample degradation before proceeding gDNA purification listed in the protocol.

Experimental Procedure

Digesting LCM Samples

1. After performing LCM capture of cells or tissues, if you use MMI LCM method, add 100 µl Digestion Buffer to the capturing tube and close the tube cap. If you use Arturus LCM method, add 100 µl Digestion Buffer to a 0.5 ml microtube (not provided) which is matched with original capturing HS Cap. Close the HS Cap tight. Invert the tube and tap the cap with a finger until the solution spreads and covers the whole cap completely.
Notes:
(1) For LCM sample from frozen tissues, use Digestion Buffer D1 (DD1) premixed with Proteinase K.
(2) For LCM sample from sperm, use Digestion Buffer D1 (DD1) premixed with Proteinase K and DTT.
(3) For LCM samples from FFPE tissues, use Digestion Buffer (DD2).
2. Incubate the inverted tube at 60°C incubator for 10 minutes to allow the captured cells and tissues detaching from the cap or polymer tabs into the digestion buffer.
3. Centrifuge briefly to collect all liquid at the bottom of the tube. Tap or vortex the tube to dispense any pellet precipitate.
4. Digestion with Proteinase K

For samples from frozen tissues, cells or from sperm: Incubate the tube at 60 °C in a thermocycler or an incubator for 3-4 hours with occasional mixing until lysis is complete. (Optional: You may perform overnight digestion at 52 °C)

For samples from FFPE tissues: Incubate the tube for 10 minutes at 94 °C, briefly spin the tube to collect all solution at the bottom of the tube. Add 10 µl Proteinase K to the tube and mix well. Incubate the tube at 60 °C in a thermocycler or an incubator for 3-4 hours with occasional mixing until lysis is complete. (Optional: You may perform overnight digestion at 52 °C).

At the end of incubation, no pellets should be visible, otherwise, extend incubation 30 minutes more to make sure no pellets exist.

5. Centrifuge the microtube at maximum speed (about 13000 rpm or 16000 X g) for one minute.

Binding DNA Products

1. Transfer all 100 µl cell lysate solution from the capturing tube to a Binding Tube (BT3).
2. Add 200 µl of Binding Buffer (PB5) to the Binding Tube (BT3). Mix well with the lysate by pipetting up and down the solution 10 times to obtain a homogenous solution.
3. Close the Binding Tube (BT3) cap.
4. Centrifuge the Binding Tube (BT3) at maximum speed (about 13000 rpm or 16000 X g) at room temperature for 5 minutes to bind the DNA. (Note: During centrifugation, always position the micro-tube hinge pointed outward from the center of rotation. Majority of DNA will collect at the bottom along the hinge side of the Binding Tube.)

5. Open the cap.
6. 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 250 µL Wash Buffer II (WB2) containing ethanol to each well. Mix by pipetting solution up and down 2 – 3 times and incubate the tube for 10 – 30 seconds at room temperature.
2. Centrifuge the Binding Tube at maximum speed (\geq 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 Washing Buffer (WB2) from the Binding Tube (BT3) using one of the methods suggested in step 6 of “Binding DNA 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 (BT3) 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 (BT3) in a lab hood for 8 – 10 minutes to remove any residual liquid.

Eluting Products

Genomic DNA attached to the wall of the Binding Tube can be analyzed directly in the same tube by restriction enzyme digestion, primer extension, SNP detection and sequencing without elution. If the gDNA is to be eluted, follow the procedure below.

1. Add 10 µL - 40 µL Elution Buffer (EB1) into the Binding Tube (BT3). (If higher concentration is preferred, add 10 µL Elution Buffer (EB1) and use Optional #1 or Optional #3 to elute gDNA product).
2. Optional #1 (Elute with vortex): Close the tube and vortex the tube for 30 seconds. Centrifuge the tube at maximum speed briefly to collect all liquid at the bottom of each tube.
Optional #2 (Elute with pipette): Pipette the solution up and down 5 – 7 times, and incubate the strip for >60 seconds at room temperature.
Optional #3 (Elute with shake): Close the tube and shake the tube for 5 – 6 minutes with a moderate-speed shaker.
3. The eluted gDNA may be used immediately in downstream applications. Alternatively, the eluted gDNA may be stored at 4 °C for short-term storage or -20 °C for long-term storage.

Electrophoresis and Downstream Application

The size of purified DNA can be examined by agarose gel electrophoresis. Quantity of purified DNA may be determined by UV absorbance at 260 nm or fluorescent DNA assay. Purified DNA in the tube can be used immediately for downstream applications, such as enzyme digestion, SNP analysis, PCR, STR analysis, DNA sequencing, whole genome amplification (WGA) and other molecular manipulations.

Troubleshooting

Problem	Cause	Solution
Low yield of product	Number of cells used was less than recommended or Poor quality of starting material Incomplete lysis Low centrifugation forces Pipette tips scrap the wall of the Binding Tube too much during aspiration of solution	Be sure the protocol for LCM cell capture, collection and processing is followed correctly. Increase the number of captured cells if possible. If incomplete lysis is observed, extend the Proteinase K digestion time for an addition of 15 – 30 minutes. Make sure the Binding Tube was spun at ~16000 x g for 5 minutes. If lower speed is used, increase the spin time. Be sure to put the pipette tip at the exact center of the bottom of the Binding Tube during aspiration.
No PCR product	Missing Component in the PCR mixture	Be sure to add all components. Check positive control and negative control for PCR reaction.