Isolation of Living *In Vitro* Cells Using the Applied Biosystems® Arcturus$^{XT™}$ Microdissection Instrument

Petri Dish Protocol

Introduction

Laser capture microdissection (LCM) is a well-established technology used by thousands of researchers to enrich sample populations for use in highly specific downstream analyses. Scientists in a number of fields, including stem cell research, desire to harness the specificity and efficiency of LCM—historically used for tissue- and slide-based applications—for use in live-cell experiments.

The Applied Biosystems® Arcturus$^{XT™}$ Microdissection Instrument provides researchers with a tool to isolate live cells from culture in a Petri dish for subsequent reculture and investigation. This application note describes an optimized method for live-cell microdissection using the entire Arcturus$^{XT™}$ Live Cell Microdissection Module, including the specialized Arcturus$^{XT™}$ Petri Dish Stage Insert, Arcturus$^{XT™}$ Live Cell Growth Chamber, and Arcturus$^{XT™}$ Microdissection Petri Dish.

Materials

- Arcturus$^{XT™}$ Microdissection Instrument (Cat. #ARCTURUSXT)
- Arcturus$^{XT™}$ Petri Dish Stage Insert (Cat. #0310-5631)
- Arcturus$^{XT™}$ Live Cell Growth Chamber, PEN membrane bottom (Cat. #5000300)
- Arcturus$^{XT™}$ Microdissection Petri Dish, silicone coated (Cat. #5000301)
- Cell culture media (Major Lab Supplier (MLS))
- Curved-tip forceps (MLS)
- 70% ethanol (VWR Cat. #34172-00)
- CO_2 incubator (MLS)
- Pipettors and sterile pipette tips (MLS)
- Kimwipes® Delicate Task Laboratory Wipers (Kimberly-Clark)
- Petri dish, sterile, 50–60 mm diameter (MLS)
- RNase AWAY® wipes (Cat. #10328-011)

Methods

Instrument Configuration

Before proceeding with microdissection, ensure that the Arcturus$^{XT™}$ instrument has been properly configured for use with the Arcturus$^{XT™}$ Petri Dish Stage Insert. Refer to the Arcturus$^{XT™}$ Petri Dish Stage Insert Installation Guide for complete instructions.

1. Install the Arcturus$^{XT™}$ Petri Dish Stage Insert and configure the Arcturus$^{XT™}$ operating software to accommodate the Arcturus$^{XT™}$ Petri Dish Stage Insert.
2. Launch the Arcturus$^{XT™}$ operating software and verify the ultraviolet (UV) laser positions at all magnifications.
   Note: Once the Arcturus$^{XT™}$ Petri Dish Stage Insert has been installed and configured, the infrared (IR) laser will be disabled and only the UV laser will be available for use.

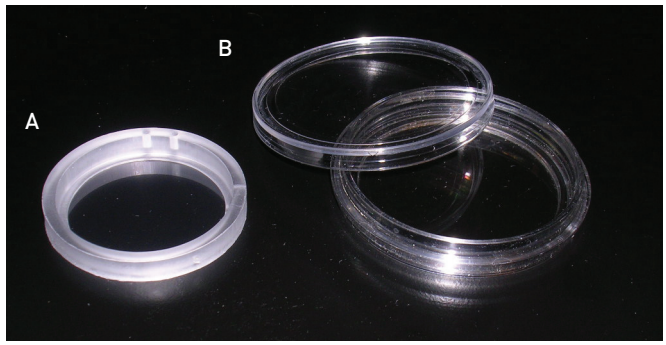

Figure 1. Arcturus$^{XT™}$ Live Cell Microdissection Consumables. A. The Arcturus$^{XT™}$ Live Cell Growth Chamber. **B.** The Arcturus$^{XT™}$ Microdissection Petri Dish.

Specimen Preparation

Important Notes:

- Perform specimen preparation under standard sterile cell culture conditions.
- The Arcturus$^{XT™}$ Petri Dish Stage Insert is intended for use with specific Petri dishes. Standard cell culture dishes may not be used with the Arcturus$^{XT™}$ Microdissection Instrument. Please refer to the Materials section for details.

1. Place a sterile Arcturus$^{XT™}$ Live Cell Growth Chamber (Figure 1A) into a sterile 50 mm or 60 mm Petri dish.
2. Seed cells onto the PEN membrane of the Arcturus$^{XT™}$ Live Cell Growth Chamber in 1–2 mL of culture media (Figure 2A).
   Note: Take care not to puncture the PEN membrane when adding the cells to the Arcturus$^{XT™}$ Live Cell Growth Chamber.
3. Add 1–2 mL of additional media to the Petri dish, exterior to the Arcturus$^{XT™}$ Live Cell Growth Chamber Insert (Figure 2B).
4. Allow cells to grow to desired confluency.
5. Obtain a sterile Arcturus$^{XT™}$ Microdissection Petri Dish and place into the culture hood.
6. Remove media from the Arcturus$^{XT™}$ Live Cell Growth Chamber containing the cultured cells and then place 200–300 µL of fresh media back into the Arcturus$^{XT™}$ Live Cell Growth Chamber.
7. Using fine-tipped curved forceps, remove the Arcturus$^{XT™}$ Live Cell Growth Chamber from the Petri dish.
   7a. Insert the forceps tips into the two small holes on the top of the ring (Figure 3A) or use the forceps to gently hold the sides of the ring (Figure 3B).
   7b. Gently lift out the Arcturus$^{XT™}$ Live Cell Growth Chamber.
8. Carefully dry the underside of the Arcturus$^{XT™}$ Live Cell Growth Chamber using a dry Kimwipe. Wipe the bottom of the dish several times until completely dry.
   Important Note: It is critical to ensure that the underside of the Arcturus$^{XT™}$ Live Cell Growth Chamber is dry. Any moisture may affect the microdissection process.
9. Place the dry Arcturus$^{XT™}$ Live Cell Growth Chamber into the Arcturus$^{XT™}$ Microdissection Petri Dish, on top of the silicone layer. Use the forceps to gently push down on the top of the plastic ring to ensure good contact between the underside of the Arcturus$^{XT™}$ Live Cell Growth Chamber and the silicone layer of the Arcturus$^{XT™}$ Microdissection Petri Dish.

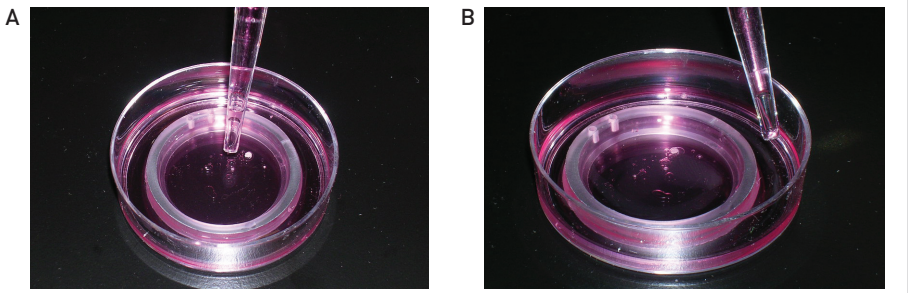

Figure 2. Cell Culture in the Arcturus$^{XT™}$ Live Cell Growth Chamber. Live cells are seeded directly onto the PEN membrane of the Arcturus$^{XT™}$ Live Cell Growth Chamber, which is placed inside a standard petri dish. Media is added to both the Arcturus$^{XT™}$ Live Cell Growth Chamber and to the surrounding petri dish to facilitate cell growth.

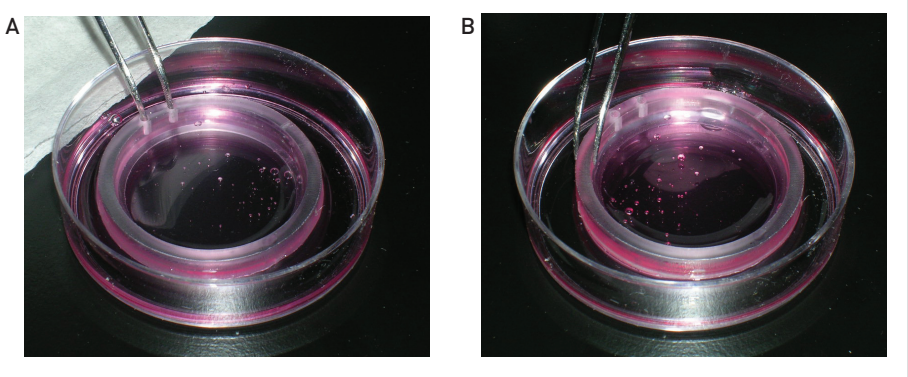

Figure 3. Removing Arcturus$^{XT™}$ Live Cell Growth Chamber from Petri Dish. Under sterile conditions, the Arcturus$^{XT™}$ Live Cell Growth Chamber is carefully removed from the standard petri dish for transfer to the Arcturus$^{XT™}$ Microdissection Petri Dish.

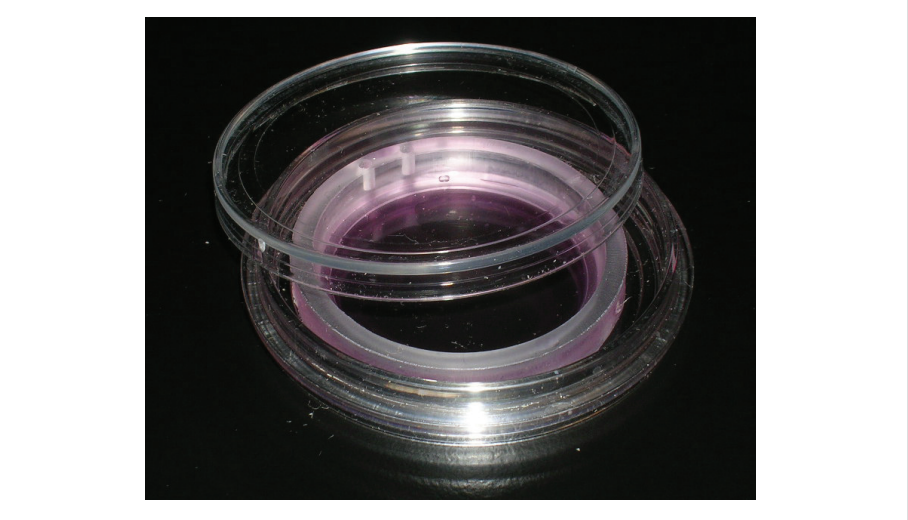

Figure 4. Arcturus$^{XT™}$ Live Cell Growth Chamber/Microdissection Dish Assembly with Media. Once the Arcturus$^{XT™}$ Live Cell Growth Chamber has been placed into the Arcturus$^{XT™}$ Microdissection Petri Dish, the entire assembly is carried to the Arcturus$^{XT™}$ Microdissection Instrument. With the lid on the Arcturus$^{XT™}$ Microdissection Petri Dish, sterility is maintained throughout the microdissection experiment.

10. Add 1 mL of fresh media to the Arcturus$^{XT™}$ Live Cell Growth Chamber only.
    Note: The Arcturus$^{XT™}$ Microdissection Petri Dish, outside of the Arcturus$^{XT™}$ Live Cell Growth Chamber, should remain dry.
11. Replace the lid onto the Arcturus$^{XT™}$ Microdissection Petri Dish (Figure 4). The lid will remain on the Arcturus$^{XT™}$ Microdissection Petri Dish throughout the microdissection experiment, maintaining sterile conditions for the live cells.

Laser Microdissection

The following settings were used for protocol validation and should be used as a guideline for the microdissection of live cells. Optimization of settings may be required, depending on the individual cell type and preparation.

UV Laser Settings

- UV Laser Power: 50% (ND Filter #1 "In"). Open upper panel on left side of instrument, and push in Filter #1 to reduce power to 50%. Verify from the label located on the inside of the door that insertion of filter #1 reduces the UV laser power by 50%.
- UV Laser Speed: 500
- UV Cut Length: 500
- Tab length: 0
- Automatic LCM spots: 0
- If necessary, adjust the UV laser focus point to optimize UV cutting conditions with the Arcturus$^{XT™}$ Microdissection Petri Dish. See the Arcturus$^{XT™}$ User Manual if this step is required.

Microdissection Protocol

1. Thoroughly clean the instrument and work area with 100% ethanol and RNase AWAY® or RNase*Zap*® wipes.
2. Load the Arcturus$^{XT™}$ Microdissection Petri Dish–Growth Chamber assembly onto the Arcturus$^{XT™}$ Petri Dish Stage Insert. Keep the lid on the Petri dish to maintain sterility (Figure 5).
3. At desired magnification, identify and mark up areas for microdissection.

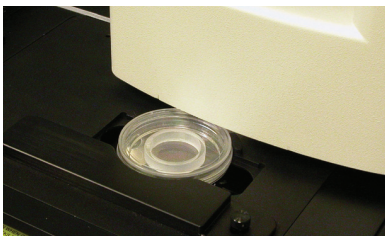

Figure 5. Arcturus$^{XT™}$ With Dish Assembly on Stage.

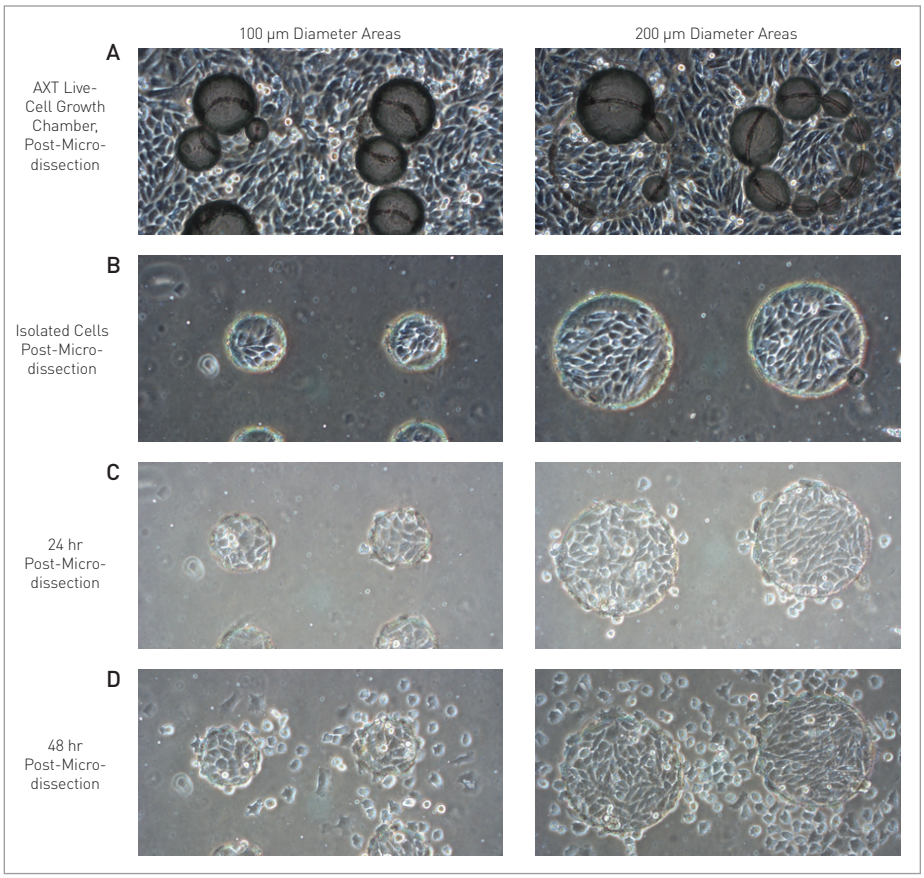

Figure 6. Arcturus$^{XT™}$ Instrument Laser Microdissection of Live Cells.

4. Click on the "UV Cut Only" button in the microdissection tool pane to activate the UV laser and cut around the marked up cells.
5. With the lid still on the Arcturus$^{XT™}$ Microdissection Petri Dish, remove the Arcturus$^{XT™}$ Microdissection Petri Dish–Growth Chamber assembly from the instrument.
6. Place the Arcturus$^{XT™}$ Microdissection Petri Dish Growth Chamber assembly into an incubator for at least 5 minutes.
7. Remove the Arcturus$^{XT™}$ Microdissection Petri Dish Growth Chamber assembly from the incubator and take it to a cell culture hood.
8. Remove the Arcturus$^{XT™}$ Microdissection Petri Dish lid, and with a pair of curved forceps, place the tips into the holes in the ring of the insert, or grasp the plastic ring while holding down the bottom of the Petri dish with your opposing hand. Then, very slowly and carefully lift the insert to remove it from the silicone layer.
9. The microdissected cells will be left behind and attached to the silicone layer.
10. Discard the Arcturus$^{XT™}$ Live Cell Growth Chamber, or:
    a. Place it into another Arcturus$^{XT™}$ Microdissection Petri Dish for additional microdissection. (Start from Step 8 of the Specimen Preparation section above.)
    or
    b. Retain for continued culturing of remaining cells and future evaluation by placing it into a 50 mm or 60 mm Petri dish. (See Sample Preparation section above.)
11. Add 1–2 mL of fresh media to the Arcturus$^{XT™}$ Microdissection Petri Dish containing the isolated cells and, if necessary, gently rotate the dish to spread the media across the bottom of the dish.

12. Replace the Arcturus$^{XT™}$ Microdissection Petri Dish lid and verify that the microdissected areas have attached to the bottom of the dish by using an inverted tissue culture microscope or by taking it back to the Arcturus$^{XT™}$ Microdissection System for inspection (Figure 6).
13. Place the Arcturus$^{XT™}$ Microdissection Petri Dish back into the incubator and allow the microdissected cells to grow.

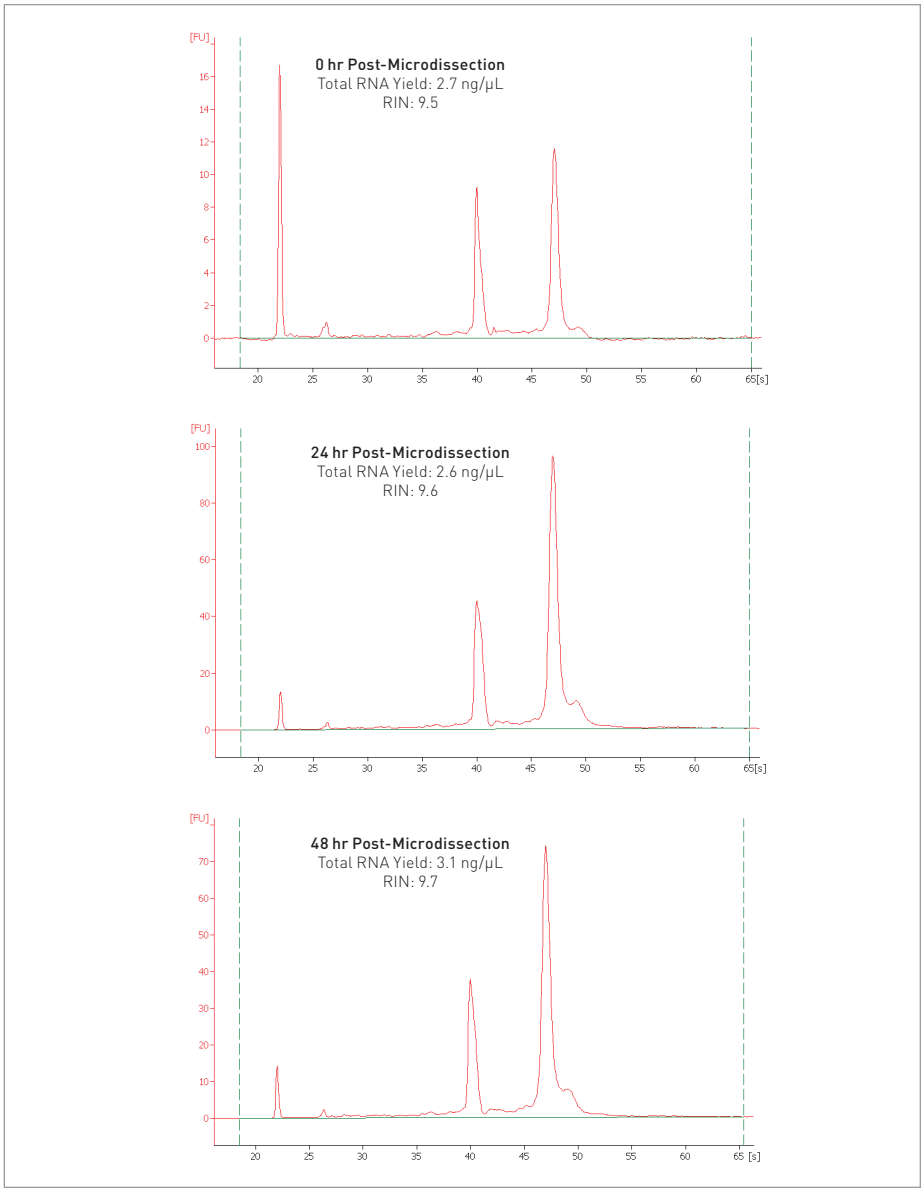

Figure 7. Total RNA Assessment, Post-Microdissection. Live cells were microdissected using the Arcturus$^{XT™}$ LCM instrument, and total RNA was isolated at 0 hr, 24 hr, and 48 hr post-microdissection. Total RNA quality (Agilent Bioanalyzer) and total RNA yield (NanoDrop® ND-1000) were measured, both demonstrating that the microdissection process does not damage the RNA.

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