LAXCO
MICROSCOPES REDEFINED

ISOLATION OF LIVING IN VITRO CELLS USING THE ACCUVA CELLECT

APPLICATION NOTE #13

INTRODUCTION

Laser capture microdissection (LCM) is a well- established technology, used by thousands of researchers to enrich sample populations for use in highly-specific down-stream 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 LCM technology found in the Accuva Cellect from Laxco provides researchers with a tool to isolate live cells from culture in a petri dish for subsequentre-culture and investigation. This technical note describes an optimized method for live cell microdissection module and stage insert included with the instrument and specialized live cell growth chamber and petri dish.

MATERIALS NEEDED

- LCM instrument Accuva Cellect (Laxco Inc. Cat# LCM-FL1A)
- Petri dish stage insert (included with instrument)
- Live Cell Growth Chamber,PEN membrane bottom (ThermoFisher Cat. #5000300)
- Microdissection Petri Dish, silicone coated (Thermo Fisher Cat. #5000301)
- Cellculture media(Major Lab Supplier(MLS)
- Curved-tipforceps(MLS)
- 70%ethanol (VWR Cat.#34172-00)
- CO2 incubator (MLS)
- Pipettors and sterile pipette tips(MLS)
- Kim wipe Delicate Task Laboratory Wipers (Kimberly-Clark)
- Petridish,sterile,50–60mmdiameter(MLS)
- RNaseAway(ThermoFisher Cat.#10328-011)
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METHODS

Instrument configuration

Before proceeding with microdissection, ensure that the LCM instrument has been properly configured for use with the petri dish stage insert. Refer to the petri dish stage insert installation guide found in the appendix of the user guide for complete instructions.

Step 1. Install the petri dish stage insert and configure the LCM operating software to accommodate the petri dish stage insert.

Step 2. Launch the LCM operating software and verify the ultraviolet (UV) laser positions at all magnifications.

Note: Once the petri dish stage insert has been installed and configured, the infrared (IR) laser will not be used, and only the UV laser will be used for the experiment. Please be sure to use only the "cut" button (not cut-and-capture) when dissecting the desired cells.

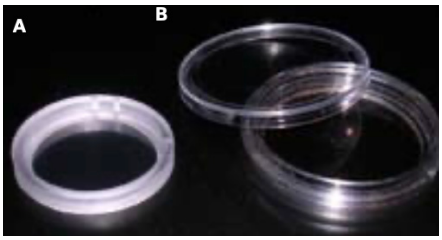
SPECIMEN PREPARATION

Important Notes:

- Perform specimen preparation under standard sterile cell culture conditions.
- The microdissection petri dish Insert is intended for use with specific petri dishes. Standard cell culture dishes may not be used with the LCM instrument. Please refer to the materials section for details.

Step 1. Place a sterile live cell growth chamber (Figure 1A) into a sterile 50 or 60 mm petri dish.

Live Cell Microdissection Consumables (Figure 1)



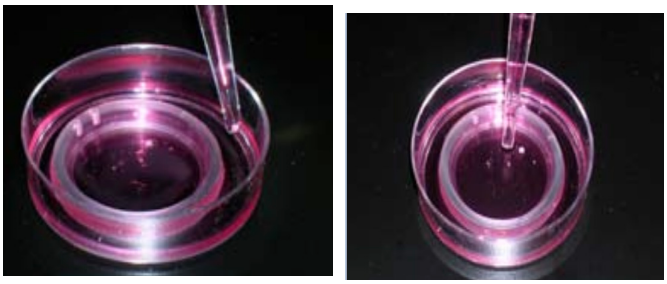
Step 2. Seed cells onto the PEN membrane of the live cell growth chamber in 1–2 mL of culture media. (See Figure 2A.)
Note: Take care not to puncture the PEN membrane when adding the cells to the Live cell growth chamber.

Step 3. Add 1–2 mL of additional media to the petri dish, exterior to the live cell growth chamber insert. (See Figure 2B.)

Step 4. Allow cells to grow to desired confluency.

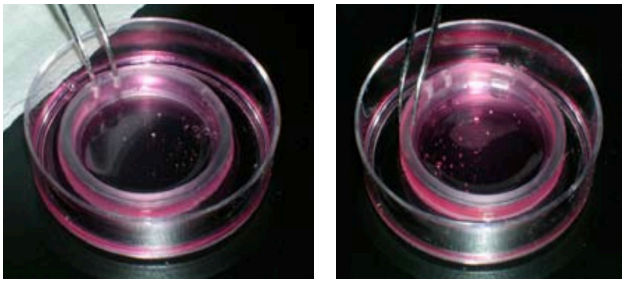
Step 5. Obtain a sterile microdissection petri dish and place into the culture hood.

Cell Culture in the Live Cell Growth Chamber (Figure 2)



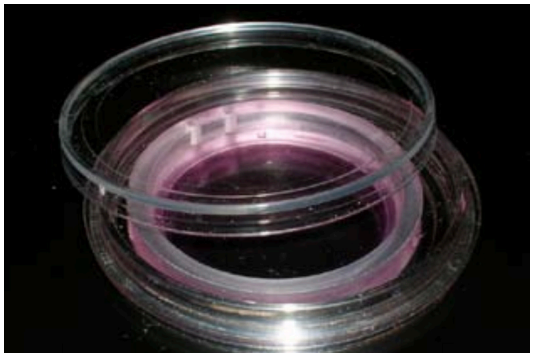
Live cells are seeded directly onto the PEN membrane of the Live cell growth chamber, which is placed inside of a standard petri dish. Media is added to both the Live Cell Growth Chamber and to the surrounding petri dish to facilitate cell growth.

Removing Live Cell Growth Chamber from Petri Dish (Figure 3)



Under sterile conditions, the Live cell growth chamber is carefully removed from the standard petri dish for transfer to the Microdissection Petri dish.

Live cell growth chamber/Microdissection Dish Assembly with Media (Figure 4)



Once the Live cell growth chamber has been placed into the microdissection petri dish, the entire assembly is carried to the LCM instrument. With the lid on the microdissection petri dish, sterility is maintained throughout the microdissection experiment.

Step 6. Remove media from the Live cell growth chamber containing the cultured cells and then place 200–300 µL of fresh media back into the Live cell growth chamber.

Step 7. Using fine-tipped curved forceps, remove the 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 live cell growth chamber.

Step 8. Carefully dry the underside of the live cell growth chamber using a dry Kimwipe. Wipe the bottom of the dish several times until completely dry.

Important Note: It is very critical to ensure that the underside of the live cell growth chamber is dry. Any moisture may affect the microdissection process.

Step 9. Place the dry live cell growth chamber into the 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 live cell growth chamber and the silicone layer of the microdissection petri dish.

Step 10. Add 1 mL of fresh media to the Live cell growth chamber only.

Note: The microdissection petri dish, outside of the live cell growth chamber, should remain dry.

Step 11. Replace the lid onto the microdissection petri dish. (See Figure 4.) The lid will remain on the microdissection petri dish throughout the microdissection experiment, maintaining sterile conditions for the live cells.

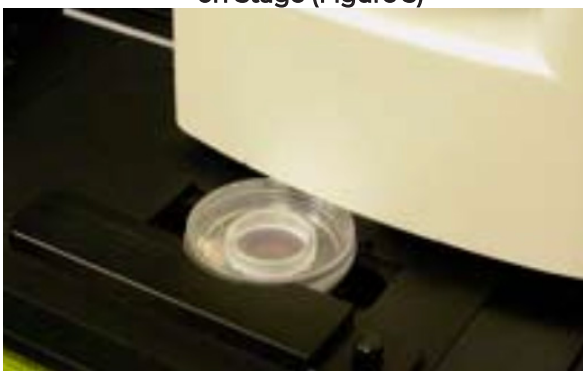
LASER MICRODISSECTION

The UV laser settings for live cell microdissection will need to be a little higher than used for standard tissue microdissection because of the energy transfer to the media. Optimization of settings maybe required depending on the individual cell type and preparation.

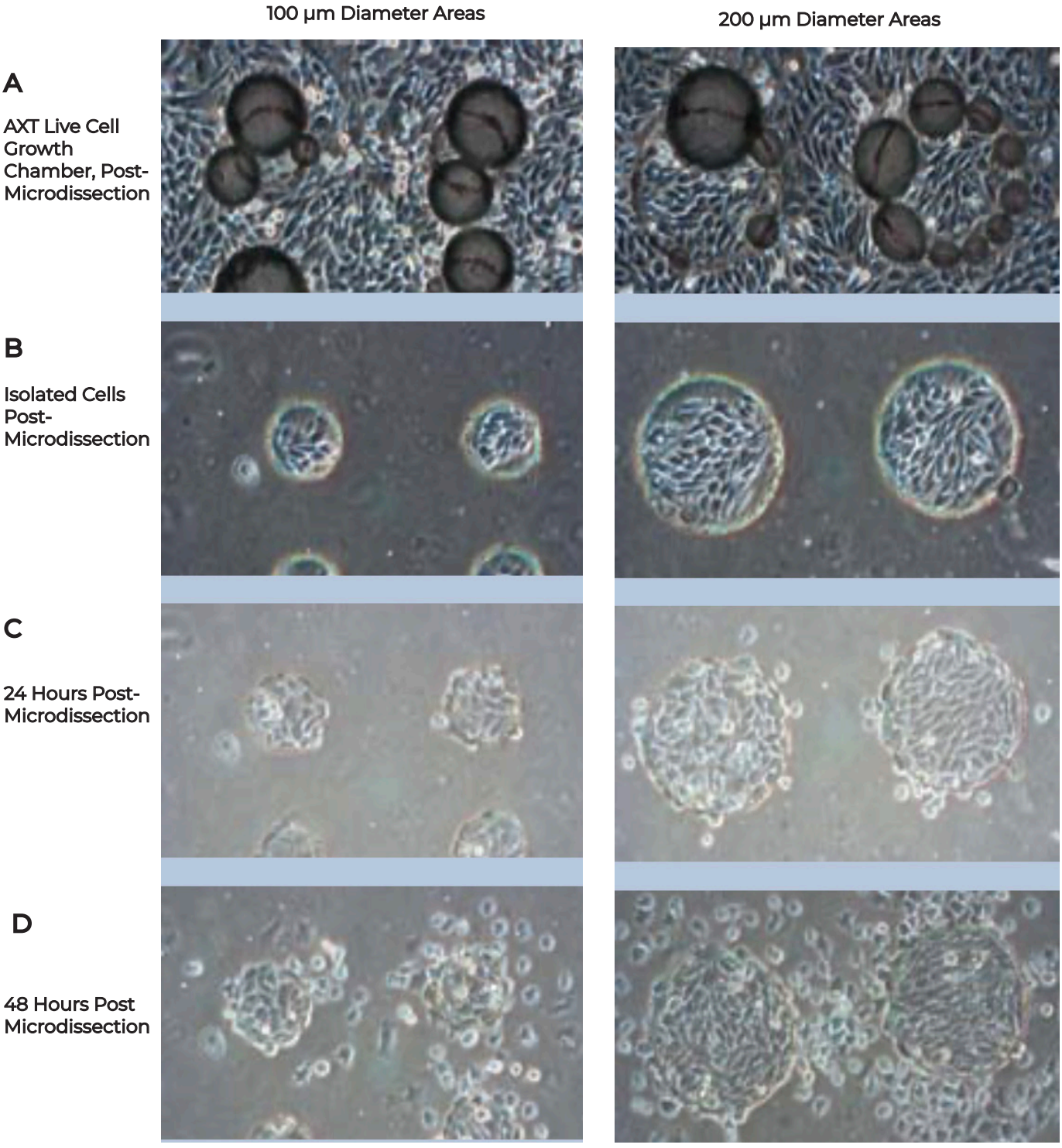
MICRODISSECTION PROTOCOL

Step 1. Thoroughly clean the instrument and work area with 100% ethanol and RNase- Away or RNase-Zap.

LCM Instrument With Dish Assembly on Stage (Figure 5)



Microdissection of Live Cells (Figure 6)



Step 2. Load the microdissection petri dish-growth chamber assembly onto the petri dish stage insert. Keep the lid on the petri dish to maintain sterility. **(See Figure 5.)**

Step 3. At desired magnification, identify and mark up areas for microdissection.

Step 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.

Step 5. With the lid still on the microdissection petri dish, remove the microdissection petri dish-growth chamber assembly from the instrument.

Step 6. Place the microdissection petri dish-growth chamber assembly into an incubator for at least 5 minutes.

Step 7. Remove the microdissection petri dish-growth chamber assembly from the incubator, and take it to a cell culture hood.

Step 8. Remove the 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 with your opposing hand, hold down the bottom of the petri dish. Then very slowly and carefully lift up the insert to remove it from the silicone layer.

Step 9. The microdissected cells will be left behind and attached to the silicone layer.

Step 10. Discard the Live cell growth chamber, or:

a. Place it into another microdissection petri dish for additional microdissection. (Start from **Step 8** of the Specimen Preparation section above.)

or

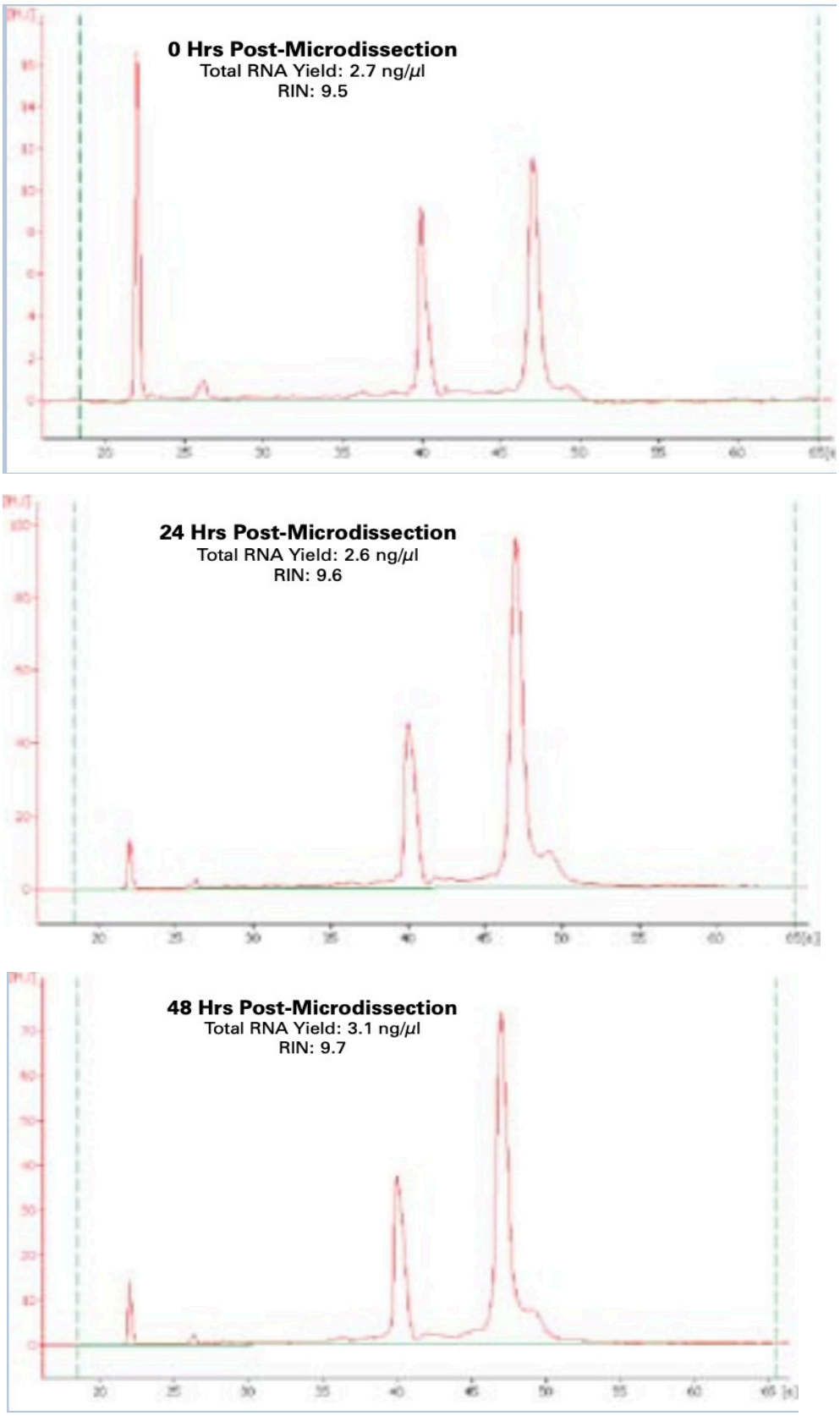
b. Retain for continued culturing of remaining cells and future evaluation by placing it into a 50 or 60 mm petri dish (See the sample preparation section above).

Step 11. Add 1–2 mL of fresh media to the microdissection petri dish containing the isolated cells, and if necessary, gently rotate the dish to spread the media across the bottom of the dish.

Step 12. Replace the 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 LCM instrument system for inspection. **(See Figure 6.)**

Step 13. Place the microdissection petri dish back into the incubator and allow the microdissected cells to grow.

Total RNA Assessment, Post-Microdissection (Figure 7)



Live cells were microdissected using the LCM instrument, and total RNA was isolated at 0 hrs, 24 hrs and 48 hrs post-microdissection. Total RNA quality and total RNA yield were measured, both demonstrating that the microdissection process does not damage the RNA.

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