

Live Cell Laser Capture Microdissection

Power: 80-84 mv

Duration: 1.50-2.00 ms

Magnification: 7.5 um (smallest spot size only)

*Power can be increased as needed without damage to cells as it is only the area around cells that is captured.

-When dehydrating the back of the slide in 100% ethanol prior to start, take care not to scratch the plastic when wiping the slide surface.

-Slide must be completely dry.

-Vacuum does not function on this type of slide material, therefore, take care when placing and removing cap.

-Remove cap from film surface gently.

-When capturing cells, leave enough room along the periphery of the cells so that the plastic does not merge with the live cells.

-Cells are difficult to observe (regardless of magnification) due to lack of staining and porous film material.

-Timing is the most important factor in cell viability; perform the capture as quickly as possible, before cells dry out (optimal time is 10 minutes).

Or

- Alternatively, keep re-hydrating the slide periodically before drying out occurs i.e every 8 minutes or so.

-Do not waste time checking cells on the cap if you are certain that cells have been captured.

Experimental Problems:

1. Drying out of cells during capture.
2. Not enough cells captured on cap to be cultured.
3. Laser has passed over the sample and burned the cells.
4. Post-Capture: transfer to ependorf/trypsinization/culturing.
5. Power setting is not high enough to pierce/detach the slide film.

Xenogen Corporation, 860 Atlantic Ave., Alameda CA 94501 USA
Phone 510.291.6100 • Fax 510.291.6196 • www.xenogen.com

Pictures (for stained +
[on LM frozen sections]
[computer])
Ziv (2004)

↓
pictures

↓
150704



XENOGEN

Discovery in the Living Organism™

Cell Passaging: *for Fibroblasts*

Take out necessary **materials**:

- Te (trypsin)
- 3 x 10 ml pipettes
- pipette gun
- HBSS or PBS for rinsing (optional)
- pipette for vacuum
- 1 x 15 ml blue cap tube
- New flask(s)
- Cell medium (DMEM + FBS 10% + ABC)

*Sterilize equipment with 70% ethanol as needed

*Open tubes ahead of time.

*Open hood vacuum

Procedure:

- 1) Warm Te, TNS, and HBSS in warm water bath
- 2) Aspirate the medium from the flask using the vacuum.. Vacuum from the corner of the flask, careful not to aspirate the cells!
- 3) Using a 10 ml pipette, add 3-4 ml of HBSS to wash the flask, and swirl gently.
- 4) Vacuum out the HBSS.
- 5) Repeat steps 3 and 4.
- 6) Add 3ml (for T75) or 1 ml (for T25) of Te to flask using same pipette.
- 7) Shake flask gently and check for cell detachment under the microscope.
- 8) Using a 10 ml pipette, add 4 ml (T25) or 12 ml (T75) of medium to the flask.
- 9) Aspirate and expel in order to homogenize, washing the surface. Do NOT make bubbles!
- 10) Aspirate the suspension and place in a 15 ml capped tube using the same pipette.
- 11) Centrifuge at 400g (3000 rpm) for 5 minutes. Careful to balance the machine!
- 12) Carefully aspirate the supernatant using the vacuum. Careful not to touch the pellet! i.e. aspirate slowly coming down the side of the tube.
- 13) Shake the 15 ml tube in order to break up the pellet. Using a 10 ml pipette, add enough media so that 15 ml can be plated on to a film slide for capture and 5 ml can be cultured in a T25 flask.
- 14) Aspirate and expel in order to dissolve the pellet.
- 15) Plate 15 ml of the suspension on to a film slide (in a petri dish) for live cell capture.
- 16) Label the flask and petri dish: cell type, name, date of passage, # of passage.
- 17) Place both in the incubator at 37 C.

*Begin live cell capture once the cells on the slide have reached confluency. i.e The following day for fibroblasts and about 4-5 days for smooth muscle cells.

Post LCM- Cell removal from Cap

- 1) Place each cap containing cells into a small ependorf tube filled with media (invert the tube so media covers the cap surface).
- 2) Place ependorf tube in a petri dish and store in incubator until needed.
- 3) Remove cap from ependorf tube and place (cell-side facing up) in a petri dish.
- 4) Add 20 ul of HBSS to each cap surface and let sit for 10 minutes at RT.
- 5) Vacuum off the HBSS.
- 6) Add 20 ul of Te/EDTA to a cap surface and let sit for 2 minutes at RT (Perform this step individually for each cap).
- 7) Use a 1000ul pipette tip to transfer Te and cells to a 96 well plate.
- 8) Add 50 ul of media to each well, as needed.
- 9) Label plate and petri dish.
- 10) Incubate overnight at 37C.

Zoe Muller

Cell Counter: optional

- 1) Touch screen.
- 2) Touch arrow on screen.
- 3) If probe is present on the left side of machine, the machine is ready to use. Otherwise, you must wait till it appears.
- 4) Put the probe in the tube containing cells.
- 5) Press the large button behind the probe.
- 6) Look at the reading under WBC count, reading is in 10 cells/ml.
- 7) Figure out concentrations needed based on readings.