

Immuno-Laser Capture Microdissection

A: Development of Immuno-LCM

- Limitation of Microdissection

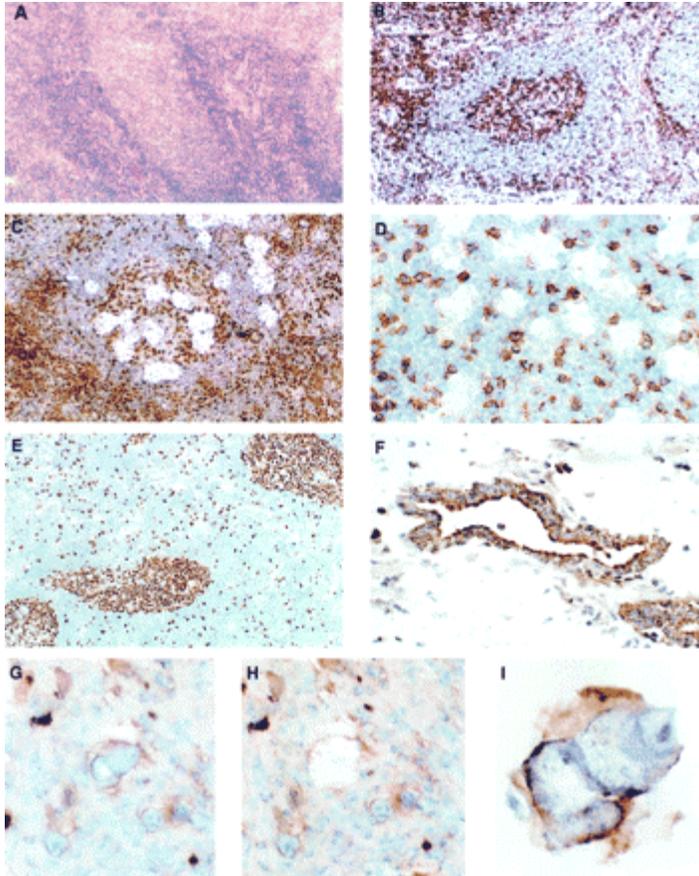
Microdissection of routinely stained or unstained frozen sections has been used successfully to obtain purified cell populations for the analysis of cell-specific gene expression patterns in primary tissues with a complex mixture of cell types. However, the precision and usefulness of microdissection is frequently limited by the difficulty to identify different cell types and structures by morphology alone.

- Immunostaining Followed by LCM

Our group, therefore, is continuing to develop rapid immunostaining procedures for frozen sections followed by laser capture microdissection (LCM) and RNA extraction. This approach allows specific mRNA analysis of cell populations which have been isolated according to their immunophenotype or expression of function-related antigens. Thus, our ability to investigate gene expression in heterogeneous tissues has been significantly expanded.

- Advantages of Immuno-LCM

- Sections fixed in acetone, methanol, or ethanol/acetone give excellent immunostaining after 12 to 25 minutes total processing time.
 - Specificity, precision, and speed of microdissection is markedly increased due to improved identification of desired (or undesired) cell types.
 - The mRNA recovered from immunostained tissue is of high quality.
 - Single-step PCR is able to amplify fragments of more than 600 bp from both housekeeping genes, e.g., beta-actin, as well as cell-specific messages, e.g., CD4 or CD19, using cDNA derived from less than 500 immunostained, microdissected cells.
- Published Example: Figures A - I ([Fend et al](#))



Figs. A & B: Frozen sections of a reactive lymph node with a central follicle, stained with H&E (A) and immunostained for CD45RO (B). Immunostaining highlights T cells within the germinal center and in the perifollicular area and makes architectural features more evident. Magnification x100.

Figs. C & D: Microdissection from germinal centers with spot sizes of 50 to 60 μm (C) and 15 to 20 μm (D) in diameter, as seen during LCM without coverslip. The smaller spot size allows avoidance of interspersed T cells. Magnification x100 (C) and x400 (D)].

Fig. E: Lymph node stained for the proliferation-associated antigen Ki67, using MIB1 antibody (dilution, 1:10) in conjunction with the Quickstain kit. Magnification x100.

Fig. F: Prostate immunostained for cytokeratin. Stromal tissue has been removed by LCM, leaving behind the positively stained glands. Magnification x400.

Figs. G to I: Microdissection of a single Reed-Sternberg cell stained for CD30 before (G) and after (H) removal. The isolated cell adherent to the membrane (I). Magnification x400 (G & H) and x1000 (I).

B: Published Protocol

This method was successful in our lab using prostate tissue and for our specific objectives. Investigators must be aware that they will need to tailor the following protocol for their own research objectives and tissue under study.

Below is the first method published by the NCI Prostate Group for Immuno-LCM. After fixation, frozen sections are immunostained under RNase-free conditions using a rapid three step streptavidin-biotin technique followed by dehydration. The immunostained sections are ready for LCM.

1. Materials

1. 1X phosphate-buffered saline, pH 7.4
2. Acetone
3. 70%, 95%, 100% ethanol
4. Xylenes, mixed ACR grade (Sigma)
5. DAKO Quick Staining kit (DAKO Corp.), a three-step streptavidin-biotin technique with prediluted mono- or polyclonal (rabbit) primary antibodies optimized for very short staining times.
6. Diaminobenzidine, DAB
7. Hematoxylin solution, Mayer's (Sigma)
8. Superfrost Plus glass slides (Fisher Scientific)
9. Placental RNase inhibitor (Perkin Elmer, Branchburg)
10. DEPC-treated H₂O (Research Genetics)

Important: Add placental RNase inhibitor to the primary antibody and the DAB solution in a concentration of 200 to 400 U/ml. All solutions are prepared with DEPC-treated water.

2. Method (under RNase free conditions)

1. Cut 8µm serial sections of snap-frozen tissue blocks on a standard cryostat with a new disposable cryostat blade.
2. Mount the tissue sections on Superfrost Plus glass slides and store immediately at -80°C.
3. Thaw the frozen sections at room temperature for 30 - 60 seconds without drying.
4. Fix by immersing immediately in cold acetone for 5 minutes.
5. Rinse the slides briefly in 1 x phosphate-buffered saline, pH 7.4.

6. Using the DAKO Quick Staining kit, immunostain the slides by incubating the slides at room temperature with the primary and secondary antibodies and the tertiary reagent for 90 to 120 seconds each, rinsing briefly with 1 x PBS between each step.
7. Develop the color with diaminobenzidine (DAB) for 3 to 5 minutes and counterstain with hematoxylin for 15 to 30 seconds.
8. Dehydrate the sections sequentially in 70%, 95%, 100% ethanol (15 seconds each) and xylene (twice for 2 minutes each).
9. Air dry.
10. The immunostained sections are then ready for LCM.

TIP: For primary antibodies other than the prediluted DAKO Quick Staining kit antibodies, the dilutions should be determined individually.

C: References

Fend F, Emmert-Buck MR, Chuaqui R, Cole K, Lee J, Liotta LA, Raffeld M. Immuno-LCM: Laser capture microdissection of immunostained frozen sections for mRNA analysis. [*Am J Pathol* 1999 Jan;154\(1\):61-6](#)

Fend F, Quintanilla-Martinez L, Kumar S, Beaty MW, Blum L, Sorbara L, Jaffe ES, Raffeld M. Composite low grade B-cell lymphomas with two immunophenotypically distinct cell populations are true biconal lymphomas. A molecular analysis using laser capture microdissection. [*Am J Pathol* 1999 Jun;154\(6\):1857-66](#)