

Preparation of lymphoid cells and tissue for molecular and immunological applications

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Equipment and reagents

- ◆ Streck Tissue Fixative (STF), molecular biology grade (Streck Laboratories, Inc.)^a
- ◆ Permeafix (Ortho Diagnostics, Inc.)
- ◆ Scalpel blades (Fisher)
- ◆ Nylon mesh, 37 μm (Fisher)
- ◆ Pestle (Fisher)
- ◆ RPMI (Gibco)
- ◆ 50 ml conical tubes (Falcon, Becton–Dickinson)
- ◆ Phosphate-buffered saline (PBS): 130 mM NaCl (Sigma), 10 mM sodium phosphate (Sigma) pH 7.4; store at room temperature
- ◆ Tissue embedding compound (Fisher)

Method

- 1 Divide the lymph node into quarters using a sterile razor blade or scalpel blade.
- 2 Wrap one quarter in aluminium foil and snap freeze in isopentane or liquid nitrogen for subsequent nucleic acid extraction.
- 3 Place another section in aluminium foil, cover in embedding compound, and snap freeze for subsequent *in situ* hybridization or RT *in situ* PCR.
- 4 Fix the third section overnight (12–24 h) in molecular biology grade STF for subsequent histology, immunohistochemistry, and PCR *in situ* hybridization.
- 5 Pass the last section through a 37 μm mesh with a pestle and sterile RPMI. Wash the cells twice in PBS: these can then be used immediately for flow cytometric analysis or resuspended in freezing medium for storage.^b
- 6 Cells for immunophenotyping cell surface antigens or intracellular antigens can be fixed in Permeafix for 1–72 h with excellent preservation of antigens and nucleic acids including mRNA.

Notes

- a STF penetrates tissue at 2 mm/h whereas 10% neutral buffered formalin penetrates at 2–4 mm/h.
- b These cells can also be used for T-cell functional assays, for example.