



Title: *Nonionic Detergent Solubilization of Lymphocytes*

No: RTLP-GL-CP9

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|----------------------|---|------------------------------------|------------------|
| •Lab coat | •Phosphate-Buffered Saline (PBS) | •Microcentrifuge | •Pipettes |
| •Gloves | •Triton-x 100 lysis buffer with protease inhibitors (4°C) | •Microfuge tubes | •Pipetteman |
| •Cultured Cells | | •Vortexer | •Pipette Aid |
| •Hemocytometer | | •10% sodium deoxy-cholate (Na-DOC) | •Pipetteman tips |
| •Light Microscope | | | •10% SDS |
| •Ice bucket with ice | | | |

This protocol has been optimized for the solubilization of the T cell antigen receptor utilizing Triton X-100 as the detergent of choice. An optional step is presented in which the ionic detergents sodium deoxycholate (Na-DOC) and sodium dodecyl sulfate (SDS) are added after removal of insoluble material and nuclei. Addition of these reagents facilitates the disruption of interactions between integral membrane proteins and is useful for the purification of individual polypeptides. Their addition, subsequent to removal of the nuclear fraction, avoids problems inherent in nuclear disruption.

Procedure: *All steps performed at 4°C or on ice*

1. Harvest cultured cells from flasks and enumerate with a hemacytometer.
2. Suspend cells in ice-cold PBS at 5×10^6 cells/ml, centrifuge 5 min at $\sim 500 \times g$, 4°C, and discard supernatant. Repeat this wash step once.

Freezing the pellet prior to lysis in step 3 may affect protein-protein interactions. In the case of the T cell antigen receptor, freezing does not adversely affect co-immunoprecipitation.

3. Resuspend the pellet in Triton X-100 lysis buffer with protease inhibitors at 10^8 cells/ml. Keep the samples 30 to 45 min at 4°C or on ice, lightly vortexing them intermittently during this time.

The ratio of cells to lysis buffer is only meant to be a guide and in most instances significant deviations will not affect the results. In cases of smaller numbers of cells ($\sim 10^7$ cells) and/or cells of large size, the cells/ml used for the lysate may need to be decreased by as much as 10-fold so that the volumes used are large

enough for practical manipulation. In the case of the T cell receptor, changing the ratio of cells to lysis buffer does not adversely affect co-immunoprecipitation of the receptor subunits; however, in systems where the lipid-to-detergent ratio is critical for maintaining chain associations, such alterations may effect co-immunoprecipitations.

It is extremely important to keep the lysate at 4 °C to decrease proteolysis and to help preserve protein-protein interactions during lysis of the cell membrane. The time required for complete lysis is variable and increases with the ratio of lipid to detergent. The addition of protease inhibitors is crucial at the time of lysis, as lysosomal and other proteases will be released. Protease inhibitors should be included throughout subsequent manipulations of the lysate.

4. Transfer sample, including insoluble material, to a 1.5-ml microcentrifuge tube. Microcentrifuge 15 min at top speed (~10,000 x g), 4°C. Carefully remove supernatant from nuclear pellet and save the supernatant. Use in conjunction with immunoprecipitation, immunoaffinity purification, or immunoblotting.

The supernatant contains solubilized membranes as well as cytosolic material. The pellet consists of nuclei, which are relatively resistant to lysis, as well as insoluble cytoskeletal elements. Care should be taken to avoid removing the insoluble fraction, especially if immunoprecipitation is to be undertaken, as this may significantly affect subsequent purification.

5. Carry out this step when disruption of protein-protein interactions is desirable (but nuclear disruption is not desirable). To the supernatant, add 10% Na-DOC to 0.2% final and 10% SDS to 0.2% final. Proceed with subsequent procedures (e.g., immunoprecipitation; see step 4).

SDS addition is performed at room temperature to prevent crystallization of SDS and DOC should be used above pH 7.5. It is important to add these additional detergents after the nuclear pellet has been removed (in step 4) to prevent release of DNA from nuclei.

Author	Management Approval/Date	Effective Date

