

Section 3B

Analysis by Immunofluorescence Microscopy

Overview of technique

Immunofluorescence microscopy with tag-specific antibodies can provide much data about the location and function of cellular proteins. For instance, the technique has been used to:

- ▶ Monitor the intracellular trafficking (movement and processing) of a transport protein in the yeast *Saccharomyces cerevisiae* (Berkower, Loayza and Michaelis, 1994)
- ▶ Clarify the structure, localization, and orientation of a membrane vesicle protein in human embryonic kidney cells (Dietzen, Hastings and Lublin, 1995)
- ▶ Determine the subcellular distribution of cDNA-encoded proteins in transfected monkey kidney cells (Algrain *et al.*, 1993)
- ▶ Localize individual domains of receptor proteins in COS-7 cells (Schöneberg, Liu and Wess, 1995)

In immunofluorescence microscopy (Figure 3B.1), a cell or tissue sample that contains the tagged protein is fixed on a slide and/or permeabilized.

The tagged protein in the sample is detected with a tag-specific antibody. The antibody detection technique may be:

- ▶ **Indirect:** The slide is incubated first with an unconjugated tag-specific antibody (primary antibody), then with a fluorochrome-conjugated antibody (secondary antibody) that recognizes the tag-specific antibody
 - ▶ **Direct:** The slide is incubated with a fluorochrome-conjugated tag-specific antibody
- Note:** Antibodies for immunofluorescent detection are conjugated with such fluorochromes as fluorescein, rhodamine, phycoerythrin, or Texas Red™.

Critical factors for success with immunofluorescence staining

The ability to immunochemically detect a tagged protein within a cell depends upon many factors. Here are some that we have found affect our experiments dramatically.

Choosing an expression system

Immunofluorescent detection of an epitope tagged protein requires adequate expression levels of tagged protein in the cell. Choose an expression vector that allows you to maximize the levels of the tagged protein without adversely affecting cell function. For more information on obtaining optimal expression of cloned proteins in mammalian cells, see, for instance, Sambrook, Fritsch, and Maniatis (1989).

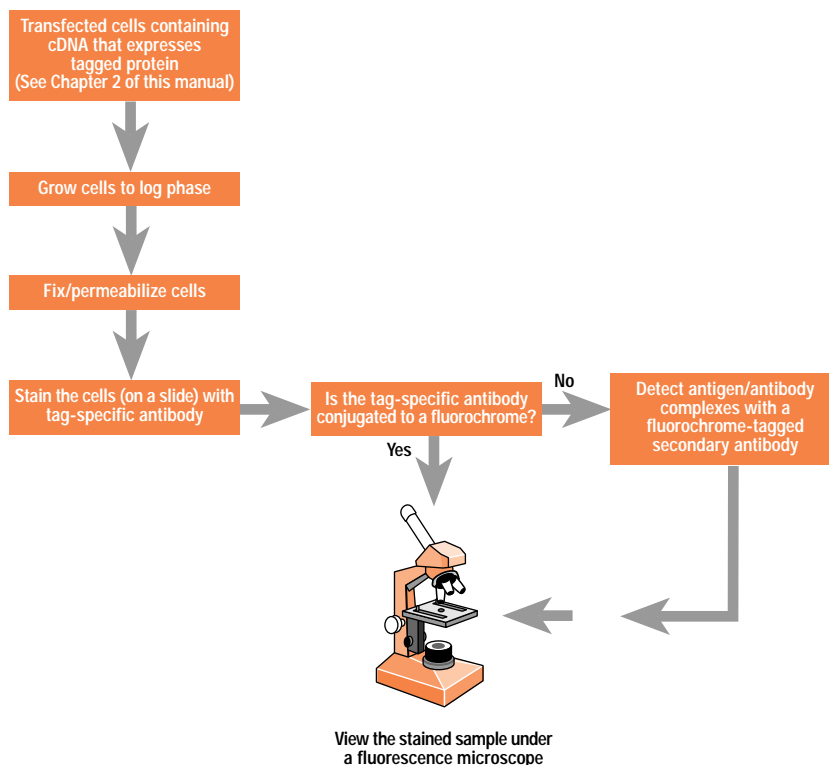


Figure 3B.1: Flow diagram for analysis of tagged proteins by immunofluorescence microscopy. Cell permeabilization allows the detecting antibody to enter the cell for analysis of antigens inside the cell. For analysis of cell surface antigens, permeabilization is not necessary.

Choosing a cell fixation method

Cell fixation is often the harshest treatment in an immunofluorescent detection procedure. The fixation techniques listed in the procedures of this section provide a good starting point, but will need to be optimized experimentally for each type of cell and specific tagged protein. To make sure that your fixation treatment will not destroy the antigen you are trying to detect, do the following:

- ▶ Consult the literature or colleagues with experience in immunofluorescent detection to be sure that the cell fixation method you use will not destroy the antigenicity of the epitope tag.
- ▶ In initial experiments, try several fixation methods to determine which gives the optimal fluorescent signal.

Fix cells differently depending upon whether the cell antigen you are detecting is located at the cell surface or within the cell. For example, some fixation methods (such as methanol or acetone fixation) are not usually used on cells that will need to be permeabilized (for examination of intracellular antigens).

Getting started: Procedures for immunofluorescence microscopy

This section describes procedures for indirect and direct immunofluorescence staining of epitope-tagged proteins in cultured mammalian cells or in yeast.

Caution: *These procedures must be optimized empirically for each individual experimental system (tagged protein, cell in which protein is expressed, tag-specific antibody, secondary antibody). Use the parameters listed in these procedures (antibody concentrations, incubation times, wash conditions, and so forth) only for initial experiments or for determining optimal ranges for each parameter. Pay particular attention to those procedural steps marked with a **Note** or **Caution** statement, since these are critical steps that must be optimized if the procedure is to produce meaningful results.*

Note: *The products indicated in colored type are available from Boehringer Mannheim. For detailed ordering information on these and related products, see the Boehringer Mannheim Product Ordering Guide, Section 5B of this manual.*

I. Detecting a tagged protein in cultured mammalian cells

Note: *This procedure is a modification of a method obtained from Dr. D.J. Dietzen of the Washington University School of Medicine. It was developed to detect a c-myc-tagged protein in stably transfected human embryonic kidney (293) cells with Anti-c-myc tag-specific antibody and goat Anti-Mouse IgG (H&L), F(ab')₂-fluorescein secondary antibody. The procedure will need to be optimized for detection of other tagged proteins or for other types of mammalian cells.*

Starting material: To perform this procedure, you must have added a suitable epitope tag sequence to a cDNA that encodes the protein to be visualized, cloned that tag-target protein construct into a mammalian expression vector (for instance, according to Algrain *et al.* [1993] or Dietzen, Hastings and Lublin [1995]), and transfected an appropriate strain of cells with the expression vector.

Note: *For more information on adding epitope tags to DNA and getting the tagged protein into cells, see Chapter 2 of this manual.*

IA. Preparing the cells

- 1 Culture the transfected cell line in an appropriate supplemented medium, using standard cell culture procedures.
- 2 Harvest the cells according to the following guidelines:

If the tagged protein is expressed in the cell	Then harvest cells
Transiently	After the cells begin expressing the tagged protein
Stably	During the exponential growth phase

- 3 Prepare cell sample in a sterile 4-chamber slide (Nunc) as follows:
 - ▶ Coat each chamber of the slide with 30 μ l of serum or poly-L-lysine (0.1 g/100 ml) solution, to ensure good cell adhesion.
 - ▶ Plate 10⁵ cells in one chamber of the slide.

Note: *Chamber slides will hold up to 4 different 1 ml cell samples. Alternatively, you can grow cells on a sterile, coated glass coverslip or slide.*
- 4 Incubate cells at 37°C overnight in standard culture media under a 5% CO₂ atmosphere.