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Section 5A

Frequently Asked Questions about Epitope Tagging

The questions below were chosen from those received by our Technical Services group.

Frequently asked questions (FAQs) about molecular tagging

1. What is the best epitope to use for tagging my protein?

A: The “best” epitope tag is the one that does not interfere with the function of the tagged protein, yet gives a strong detection signal on Western blots. There is no single “best” epitope for every tagging application, and no one can reliably predict how a particular tag will behave in any given protein. However, as a starting point for determining the right tag sequence for your particular experimental system, you can do the following:

- ▶ Do a review of the literature to see if proteins similar to yours have been successfully tagged with a particular epitope.

Note: We provide an extensive listing of epitope tag publications in Section 5C.

- ▶ Consider the copy number of the epitope tag. Multiple copies of the tag sequence may give a stronger detection signal, yet be more likely to interfere with the function of your protein.

- ▶ Consider the location of the tag. Attaching a tag to one end of a protein may be less likely to interfere with protein function, yet give an optimal detection signal.

2. Does Boehringer Mannheim have a vector that contains the c-myc, HA, or VSV-G epitope?

A: No, not at the current time. However, many labs have such vectors containing the c-myc or the HA epitope. You might try getting a suitable vector from your colleagues or from central cell repositories such as the American Type Culture Collection (ATCC). For more information, see Section 2D of this manual.

3. Will the epitope tag interfere with the function of my protein?

A: That has to be determined empirically. However, it is possible that the insertion of multiple copies of the epitope tag may interfere with protein function more than insertion of a single copy. (See also the answer to Question 1 above.)

4. Will the epitope tag cause conformational changes when it is attached to my protein?

A: This has to be determined empirically. See the answers to Questions 1 and 3 above.

5. Will the epitope tag interfere with production of my protein?

A: Generally, no.

FAQs about the Western blot procedure

1. What causes the “ghost bands” on my Western when I use anti-HA antibody as a primary antibody in an indirect detection procedure?

A: Probably nonspecific binding of either the primary or the secondary antibody conjugate to other proteins in the sample (Field *et al.*, 1988). Switching to a direct detection procedure with Anti-HA-peroxidase as the detecting antibody should eliminate the ghost bands caused by the secondary antibody conjugate, since it eliminates the need for a secondary antibody.

Also, it is always a good idea to include a negative control cell extract, prepared from the host organism but lacking the HA-tagged protein, on the Western blot. With this control, you may be able to distinguish a true positive signal from a nonspecific signal.

2. Are the epitope tagging systems from Boehringer Mannheim the most cost effective tagging system I can use for Western blots?

A: They can be, since we offer peroxidase-conjugated antibodies for direct detection of proteins tagged with c-myc, HA, or VSV-G. With the peroxidase conjugate, there are no second antibodies to buy and you save time and effort on every Western (since the direct procedure requires fewer steps than indirect procedures).

Also, the c-myc, HA, and VSV-G tagging systems have given good results in many experimental systems (as an examination of the literature will show; see, for instance, Section 1A of this manual). That means you can use the experience of others to design your own tagging experiment and have a higher chance of getting good results the first time.

FAQs about affinity chromatography

1. What is the best chemical technique for attaching this antibody to a support matrix? What type of resin will I want to use to link the antibody to? Which active group will be most advantageous?

A: Each of these questions has to be answered empirically for a given antibody. Generally, a support matrix should have a spacer arm (to make the antibody more accessible to the antigen) and form a stable linkage with the antibody (that is, it will not release antibody during harsh sample elution conditions) without interfering with the antibody binding site. See Section 4C of this manual for details.

2. What buffer should I use to dissociate the tagged protein from the antibody affinity resin without denaturing it?

A: If you prefer non-denaturing conditions, compete the tagged protein off with the corresponding tag peptide. Alternatively, genetically engineer an enzymatic cleavage site next to the tag sequence, so the target protein can be removed from the tag sequence with an eluant containing the cleaving enzyme.

In each case, of course, you must determine the best elution conditions empirically. See Section 4C of this manual for details.

FAQs about immunoprecipitation

1. Will immunoprecipitation of my tagged protein cause it to lose function?

A: The immunoprecipitation conditions listed in Section 4D will generally not cause the precipitated protein to lose function.

Note: You can increase your chances of recovering a functional protein by using the gentlest cell lysis procedure possible and by including a cocktail of protease inhibitors (as described in the Section 4B) throughout the immunoprecipitation procedure. Harsh lysing reagents and proteases are leading causes of function loss.

2. Which is better for immunoprecipitation: a secondary antibody or protein A/protein G-agarose?

A: Protein A- and protein G-agarose are most frequently used to capture antigen-antibody complexes because they bind a wider variety of primary antibodies and because they are easier to use. However, if the Fc portion of your precipitating antibody will not bind to either protein A or protein G, use a secondary antibody instead.