

## Chapter Four: Co-immunoprecipitation

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### Overview of co-immunoprecipitation

One of the most common and rigorous demonstrations of protein:protein interaction is the co-immunoprecipitation of suspected complexes from cell extracts. Co-immunoprecipitation confirms interactions utilizing a whole cell extract where proteins are present in their native conformation in a complex mixture of cellular components that may be required for successful interactions. In addition, use of eukaryotic cells enables post-translational modification which may be required for interaction and which would not occur using prokaryotic expression systems.

In a typical experiment cells are lysed and a whole cell extract is prepared under nondenaturing conditions. It is critical to use non-denaturing conditions in order to maintain any interactions that occur. An antibody specific to the bait is then added to the extract, forming a new complex. This protein:protein complex is then immobilized on protein A or protein G sepharose beads. Proteins that do not bind are removed by a series of washes. The protein complex is then eluted from the beads and dissociated by SDS sample buffer. Samples are then evaluated by SDS-PAGE followed by Western blotting with specific antibodies for the bait or prey partners.

# CHAPTER 4

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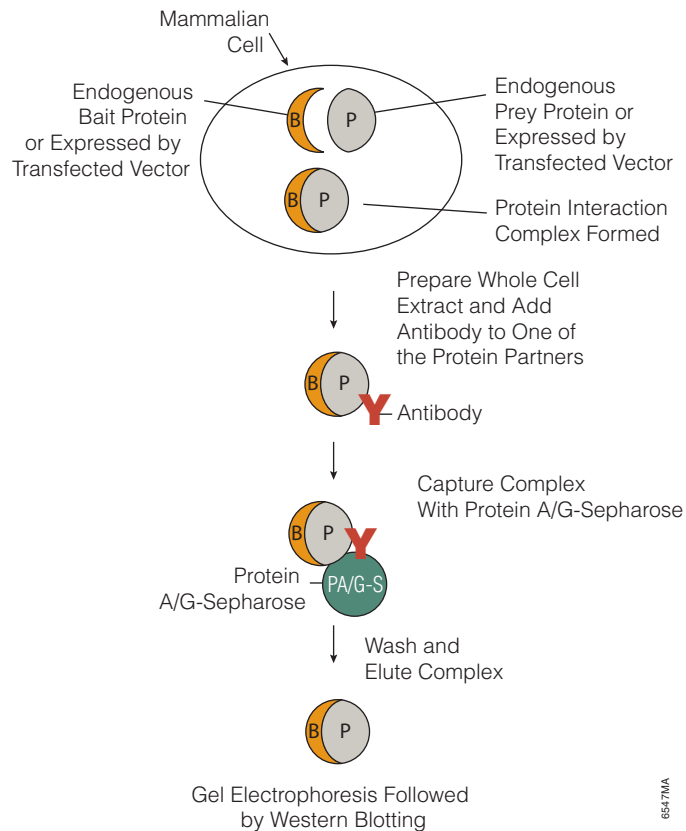


## Co-immunoprecipitation formats

### Mammalian cell-based formats (Figure 7)

Mammalian cell-based formats allow characterization of interacting partners using endogenously expressed proteins. The individual proteins should express well and antibodies should bind under non-denaturing conditions. This approach avoids the overexpression of proteins that can occur when using recombinant expression vectors. It also allows detection of native sublocation and post-translational modification, and eliminates possible issues associated with use of foreign linker and tag sequences.

If antibodies are not available or endogenous levels of individual proteins are very low, then recombinant vectors that contain short tags and encode for the relevant proteins may be transfected into the appropriate cell line. These tags (e.g., Myc) are recognized by commercially available monoclonal antibodies and can be incorporated either at the carboxyl or amino terminus of the bait or prey proteins.



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### Endogenously expressed bait and prey proteins in mammalian cells

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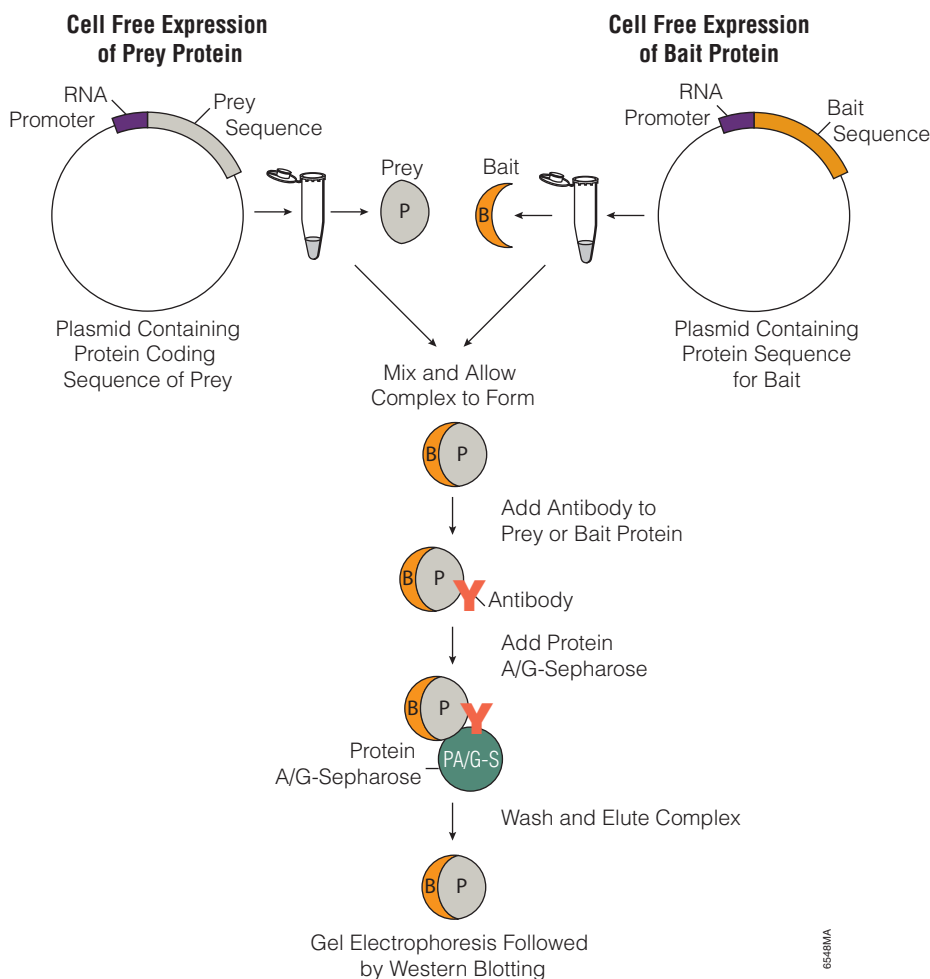


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#### Mammalian cell-free expression based formats (Figure 8)

Mammalian cell-free expression systems may also be used for co-immunoprecipitation. This method avoids the need for transfection and the generation of whole cell extracts, yet enables proteins to be expressed in a eukaryotic environment. Prey and bait partners can be expressed individually or together in a single reaction. One of the proteins can be labeled with [<sup>35</sup>S], fluorescent tagged tRNAs or both partners can be unlabeled.

As with cell-based experiments, complexes form and appropriate antibodies to either the bait or prey are added. This complex is then immobilized on Sepharose beads that are coupled to protein A or protein G. Non-specifically bound proteins are washed away from the complex. The partners are then analyzed by gel electrophoresis, followed by autoradiography or Western blotting.



**Figure 8. Schematic of immunoprecipitation using cell-free expression systems.** Coding sequences for the bait and prey proteins are cloned into individual vectors which contain the necessary elements for expression in mammalian-based cell-free systems. Following expression, aliquots from each reaction are mixed and the complex allowed to form. Antibodies to either partner are added and the complex is then captured using protein A/G-sepharose. After washing to remove non-specific proteins, the bait and prey proteins are eluted and analyzed by Western blotting using antibodies to either partner. Using an alternative procedure, either the prey or bait may be expressed as [<sup>35</sup>S] or fluorescent-labeled protein and then detected directly in the gel.

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### When to use co-immunoprecipitation

Since co-immunoprecipitation can utilize prey and bait proteins expressed in several different ways, either endogenously in mammalian cells or from cell-free expression systems, the technique can be utilized as either a discovery or a characterization tool. Use of cell-free systems for the expression of both prey and bait proteins is ideal for the rapid characterization of interactions using various mutant proteins to map domains required for interactions.

### Reagent requirements for mammalian cells as source of prey/bait proteins

- Appropriate cell line that expresses prey and bait proteins or mammalian expression vectors expressing bait and prey proteins
- Cell culture reagents
- Transfection reagent (if using recombinant vectors for expression)
- Sepharose Protein A or G
- Antibodies to bait and prey proteins or antibody to tag present in the expression vector
- Western blotting reagents

### Reagent requirements for co-immunoprecipitation using cell-free expression systems as source of prey/bait proteins

- Cell-free expression system
- Cell-free expression vectors encoding sequences for both bait and prey protein
- [<sup>35</sup>S] methionine (if expressing labeled prey)
- Sepharose Protein A or G
- Primary antibodies to either bait and prey proteins (if using unlabeled prey)
- Western blotting reagents

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### Co-localization overview

Methods such as co-immunoprecipitation and pull-downs require the preparation of cell extracts which may not preserve the physiological conditions under which proteins may interact in a true cellular environment. Using various co-localization techniques protein:protein interactions may be characterized directly in the cell without the need to create cell lysates or isolate complexes from a cell.

# 5 CHAPTER

## CO- LOCALIZATION/ FRET/BRET

