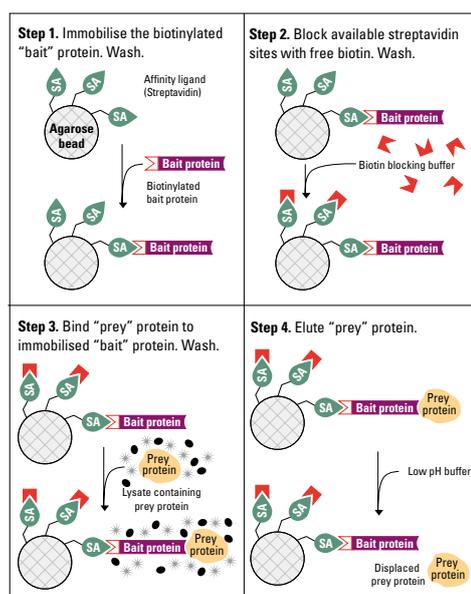


Pull-down assays

The pull-down technique is an invaluable tool for studying cellular pathways via protein interactions. The pull-down assay is an *in vitro* method used to determine physical interaction between two or more proteins. Pull-down assays are useful for measuring the conditions in which known proteins are expressed and interact and for screening to identify as yet unknown protein interactors. The minimal requirement for a pull-down assay is the availability of a purified and tagged protein (the bait) to capture and 'pull-down' a protein-binding partner (the prey).

Pull-down vs. immunoprecipitation

Pull-down assays are a form of affinity purification and are similar to immunoprecipitation except that a bait protein is used instead of an antibody. In a pull-down assay, a tagged bait protein is captured on an immobilised affinity ligand specific for the tag, thereby generating a 'secondary affinity support' for purifying other proteins that interact with the bait protein. The secondary affinity support of immobilised bait can be incubated with a variety of protein samples that contain putative prey proteins. If the buffer and sample conditions are compatible with the target binding interaction and the bait protein is functional in its tagged and immobilised state, then prey proteins present in the sample can bind to the affinity support. If the affinity of the specific binding interaction is sufficiently strong, non-bound sample components are washed away so that the prey or bait:prey-complex can be eluted from the support in purified form.



Thermo Scientific Pierce biotinylated protein interaction pull-down kit protocol.

Biotinylated protein interaction pull-down kit, Thermo Scientific Pierce

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Captures and purifies protein interactions using biotin labelled proteins.

Applications

- Study protein:protein interaction using an immobilised biotinylated bait and prey captured from a cell lysate
- Confirms a putative interaction with a prey protein captured from a cell lysate or with a previously purified prey protein
- Extracts protein:protein interaction information from *in vitro* transcription/translation lysates
- Adaptable to single or multiple sample demands
- Flexible pull-down format
- Detects a potential binding partner of a biotinylated bait protein in one day

The Thermo Scientific Pierce biotinylated protein interaction pull-down kit contains the necessary components to capture and purify interacting proteins involving a biotin labelled protein. You provide a purified, biotinylated protein as the "bait" and the cells expressing the putative protein interaction target ("prey"). The pull-down kit provides everything else: cell lysis buffer, microcentrifuge spin columns, streptavidin agarose resin, optimised binding and elution buffers, and a detailed protocol.

Catalogue No	Description
PN21115	Biotinylated protein interaction pull-down kit Sufficient materials for conducting 25 pull-down assays using a purified and biotinylated protein as the bait. Includes: Streptavidin Agarose resin, 1.5mL Tris buffered saline, 500mL Biotin blocking buffer, 15mL Wash buffer, 100mL Elution buffer (pH2.8), 50mL Spin columns, x 27 Microcentrifuge tubes, 200 x 2mL