Dynabeads® for protein complex isolation

Studying the way proteins function and interact is an exciting area of research. By looking at protein structures, researchers can identify how cofactors and other molecules influence enzymatic or protein activity.

Isolating complete protein complexes has always been problematic because the weak, ionic interactions between proteins can easily be destroyed by mechanical stress. In this application note, we discuss how to pull down intact protein complexes using Dynabeads® magnetic separation technology.

Traditional methods

Existing techniques for isolating proteins include pulldown with Sepharose® beads and spin columns, but these methods have disadvantages. With Dynabeads®, you can skip unnecessary steps that can cause your protein complexes to dissolve, including:

- Exposure to large surfaces
- Mechanical strain (e.g., centrifugation)
- Dilution
- Excessive handling (preclearing)

Although some researchers choose to preclear using Sepharose® beads, there can be nonspecific binding interactions that can contaminate the final product.

The Dynabeads® method

Add your specific antibody or interacting protein with tags to Dynabeads®, then immunoprecipitate your protein of interest. Once the beads are exposed to a magnet, they are efficiently drawn to the tube wall, taking only your protein complex with them (Figure 1). As the process is gentle, yet very quick, complexes remain intact and functional. The complexes can be resuspended in a small volume ready for downstream analysis with mass spectrometry, gels, etc. Advantages of Dynabeads® include:

- Quick and easy pulldown of intact, functional complexes
- No time-consuming preparation steps
- Only isolate the proteins you want
- Can be adapted for high-throughput applications

There is a range of Dynabeads® to choose from depending on your antibody or protein. Dynabeads® ready-coated with Protein A or Protein G are available, or you can couple your own antibodies or proteins with tags to beads such as Dynabeads® M-270 Epoxy or Dynabeads® Streptavidin.

Below are some examples of how Dynabeads® have been used in published articles to pull down protein complexes.

Featured papers

Cristea et al. used a single green fluorescent protein (GFP) tag to visualize proteins and their interactions in live cells using immunoaffinity purification with Dynabeads® M-270 Epoxy and anti-GFP antibodies. They showed that purification was rapid and efficient, and that protein complexes were in their original state with minimal nonspecific interactions. They predict that the method will help researchers understand many cellular processes.

Dynabeads® M-270 Epoxy were incubated with anti-GFP polyclonal antibodies or IgG and added to the soluble cell lysate fraction. These antibody-coated Dynabeads® bound to the target proteins and when the tube was placed in a magnet, were drawn to the tube wall. After washing, the isolated protein complexes were eluted from the beads, frozen with liquid nitrogen, and left to dry overnight by vacuum centrifugation. The pellet was put in SDS-PAGE buffer, separated on a precast 1-D gel before being stained with Coomassie® blue, prior to mass spectrometry.

This protocol can be applied to sample materials such as viruses, bacteria, yeast, mammalian tissue, cultured cells, and mice.

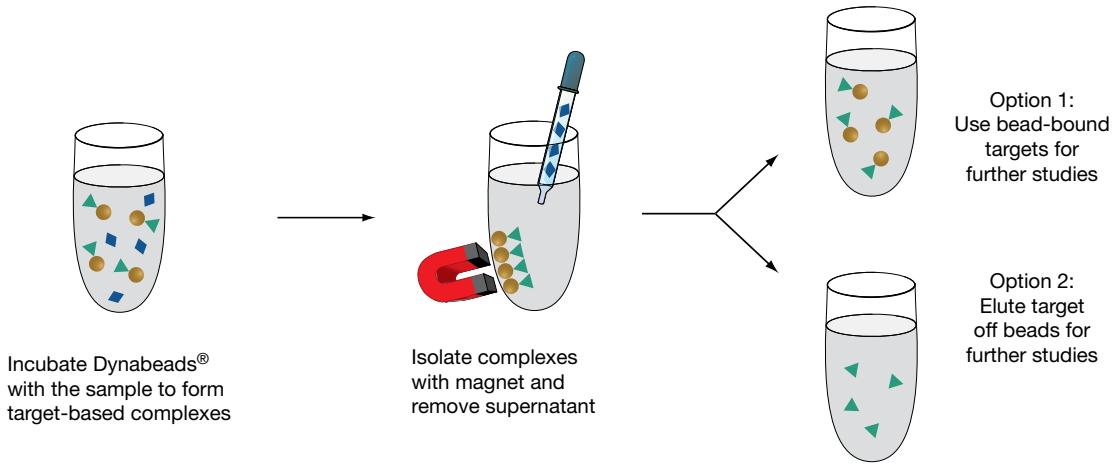

Figure 1—Isolation of protein complexes using Dynabeads®.

Devos, et al. wanted to study the nuclear pore complex (NPC), the gate that mediates traffic of macromolecules across the nuclear envelope. They used computational and biochemical means to analyze the seven proteins in one of the subcomplexes in the NPC. As part of the study, they performed proteolytic mapping of domain boundaries and loop locations in the seven yeast extract nups, the set of proteins that make up the NPC. The Protein A–tagged nups were bound to Dynabeads® M-270 Epoxy with proteolytically resistant tags and purified from yeast extracts with magnetic separation. Endoproteinases were added to hydrolyze peptide bonds and any proteolytic fragments on the beads were separated by SDS-PAGE. Cleavage sites were determined by amino-terminal Edman sequencing or by estimating the molecular weight of the fragments.

Khalili, et al. were investigating prion diseases and how the normal cellular prion protein PrP is converted into an abnormal isoform (PrPSc) in prion-diseased brains. They produced monoclonal antibodies (mAbs) for immunoprecipitation (IP) of PrPSc and the glycoforms from diseased and normal brain homogenates. Direct IP was performed using mAbs crosslinked to Dynabeads®, mouse IgG on Dynabeads® Protein A, or biotinylated antibodies with Dynabeads® M-280 Streptavidin. Indirect IP was used to capture antibody-antigen complexes with anti-PrP mAbs IgG and Dynabeads® Protein G. They demonstrated that the differentially glycosylated native PrPSc are closely associated and immunoprecipitate together.

Suzuki, et al. developed an *in vitro* pulldown assay that uses *in vitro* biotinylated proteins rather than tagged proteins as pulldown drivers with Dynabeads® Streptavidin. They synthesized the biotinylated proteins by *in vitro* transcription-translation with biotin-lysine transfer RNA. They identified the following advantages:

- No need to prepare plasmids for the pulldown drivers
- Expressed proteins are likely to be soluble
- Random position of biotin-labelled lysine residues throughout the protein mean that the fused tag does not interfere with interactions
- Biotinylated proteins are functional in many cases and maintain their native conformations

Products

Product	Volume	Cat. no.	Choose for:
Dynabeads® Protein A	1 ml	100.01D	For use with: • Human IgG 1, 2, 4 • Mouse IgG2a, 2b, 3 • Rat IgG2c • Bovine IgG2 • Canine IgG • Goat IgG2 • Guinea Pig IgG • Monkey IgG • Porcine IgG • Rabbit IgG • Sheep IgG2
	5 ml	100.02D	
Dynabeads® Protein G	1 ml	100.03D	For use with: • Human IgG 1, 2, 3, 4 • Mouse IgG1, 2a, 2b, 3 • Rat IgG2a, 2c • Bovine IgG1, 2 • Goat IgG1, 2 • Guinea Pig IgG • Horse IgG • Monkey IgG • Porcine IgG • Rabbit IgG • Sheep IgG1, 2
	5 ml	100.04D	
Dynabeads® M-270 Epoxy	60 mg	143.01D	For gentle binding of structurally intact and active peptides, proteins, and enzymes—hydrophilic surface.
	300 mg	143.02D	
Dynabeads® M-280 Tosylactivated	2 ml (30 mg/ml)	142.03D	For easy coupling of antibodies for affinity capture of proteins—hydrophobic surface.
	10 ml (30 mg/ml)	142.04	
	10 ml (100 mg/ml)	301.01	
Dynabeads® M-280 Streptavidin	2 ml	112.05D	For use with biotinylated proteins. For general protein purification, sequence-specific DNA/RNA capture, and biopanning. Needs BSA blocking.
	10 ml	112.06D	
	100 ml	602.10	
Dynabeads® MyOne Streptavidin T1	2 ml	656.01	Ideal for manual or automated protocols—low-charged and neutral beads optimal for proteins, peptides, and antibodies.
	10 ml	656.02	
	100 ml	656.03	
Dynabeads® M-280 Sheep anti-Mouse IgG	2 ml	112.01D	For use with mouse IgG1, IgG2a, and IgG 2b, not IgG3. Fc reactive.
	10 ml	112.02D	
Dynabeads® M-280 Sheep anti-Rabbit IgG	2 ml	112.03D	For use with any rabbit IgG antibody.
	10 ml	112.04D	

Featured papers:

1. Cristea, I.M. et al. (2005) Fluorescent Proteins as Proteomic Probes. *Molecular & Cellular Proteomics* 4(12): 1933–1941.

2. Devos, D. et al. (2004) Components of coated vesicles and nuclear pore complexes share a common molecular architecture. *PLoS Biol* 2(12): e380.

3. Khalili-Shizari, et al. (2005) PrP glycoforms are associated in a strain-specific ratio in native PrPSc. *J General Virology* 86: 2635–2644.

4. Suzuki, H. et al. (2004) In vitro pull-down assay without expression constructs. *BioTechniques* 37 (6): 918–919.

Other relevant articles:

1. Aartsen, W.M. et al. (2006) Mpp4 recruits Psd95 and Veli3 towards the photoreceptor synapse. *Human Molecular Genetics* 15 (8): 1291–1302.

2. Blethrow, J. et al. (2007) Modular mass spectromic tool for analysis of composition and phosphorylation of protein complexes. *PLoS ONE* 2 (4): e358.

3. Catrein, I. et al. (2005) Experimental proof for a signal peptidase I like activity in Mycoplasma pneumoniae, but absence of a gene encoding a conserved bacterial type I SPase. *FEBS Journal* 272: 2892–2900.

4. Cristea, I.M. et al. (2006) Tracking and elucidating alphavirus-host protein interactions. *J Biol Chem* 281: 30269–30278.

5. Drouet, J. et al. (2006) Interplay between Ku, Artemis and the DNA-dependent protein kinase catalytic subunit at DNA ends. *J Biol Chem* 281: 27784–27793.

6. Gudz, T.I. et al. (2006) Glutamate stimulates oligodendrocyte progenitor migration mediated via an alphav integrin/myelin proteolipid protein complex. *J Neuroscience* 26 (9) : 2458–2466.

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8. Harrison, M. et al. (2006) Some C. elegans class B synthetic multivulva proteins encode a conserved LIN-365 Rb-containing complex distinct from a NuRD-like complex. *Proc Natl Acad Sci, USA* 103(45) : 16782–16787.

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10. Kantardzhieva, A. et al. (2006) MPP3 is recruited to the MPP5 protein scaffold at the retinal outer limiting membrane. *FEBS Journal* 273: 1152–1165.

11. Kawai, T. et al. (2006) Translational control of cytochrome c by RNA-binding proteins T1A-1 and HuR. *Molecular and Cellular Biology* 26 (8):3295–3307.

12. Maeda, Y. et al. (2006) PARP-2 interacts with TTF-1 and regulates expression of surfactant Protein-B. *J Biol Chem* 281 (14): 9600–9606.

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14. Ogawa, C. et al. (2007) Gemin2 plays an important role in stabilizing the survival of motor neuron complex. *J Biol Chem* 282 (15) : 11122–11134.

15. Pinsky, B.A. et al. (2006) Glc7/protein phosphatase 1 regulatory subunits can oppose the Ipl1/Aurora protein kinase by redistributing Glc7. *Molecular and Cellular Biology* 26 (7) 2648–2660.

16. Reimers, K. et al. (2006) Sequence analysis shows that Lifeguard belongs to a new evolutionarily conserved cytoprotective family. *Int J Molecular Medicine* 18: 729–734.

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18. Schulze, W.X. et al. (2005) Phosphotyrosine interactome of the ErbB-receptor kinase family. *Mol Syst Biol* 1: 2005.0008.

19. Usui, K. et al. (2005) Protein-protein interactions of the hyperythermophilic archaeon Pyrococcus horikoshii OT3. *Genome Biology* 6:R98.

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