

Dynabeads[®] Antibody Coupling Kit

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Rev. 000

 **invitrogen**[™] | **DYNAL**[®]
invitrogen bead separations

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1. PRODUCT DESCRIPTION

1.1 INTENDED USE

The Dynabeads and buffers provided in this kit will enable you to covalently immobilize antibodies (or other protein ligands such as lectins, functional enzymes, etc.) of your choice onto the surface of Dynabeads. Antibody-coupled Dynabeads can then be used for downstream assays and experiments. Once the coupling reaction has been completed the resulting Dynabeads surface will exhibit ultra-low background binding. It is therefore not necessary to block the bead surface prior to use. ***For best results, please read through the manual carefully prior to start.***

1.2 PRINCIPLE

Antibodies (Ab) (or other protein ligands) of your choice are covalently coupled to the provided Dynabeads. Once antibodies have been coupled to the Dynabeads, the antibody coupled Dynabeads may then be used for immunoassays, immunoprecipitation, co-immunoprecipitation of protein complexes, co-immunoprecipitation of protein-nucleic acid complexes, as well as many other downstream applications. Other downstream assays could include those requiring proteins such as lectins, enzymes, or others to be coupled to the Dynabeads in stead of antibodies.

Captured proteins, protein complexes and protein-nucleic acid complexes are easily separated from lysate using magnetic separation properties of Dynabeads. Magnetic separation facilitates washing, buffer changes, and elution.

Antibody-to-bead coupling works optimally with purified antibodies, although the coupling reaction also works well with antibodies in storage buffers that include protein additives (e.g. BSA) and/or sodium azide (NaN₃). **This kit is not recommended for use with antibodies that have been stabilized in glycerol (See Section 2.3 for more information).**

Other protein ligands (e.g. lectins, enzymes, etc.) can be covalently coupled to the surface of Dynabeads using the same beads, buffers and protocol provided in this kit.

1.3 MATERIALS SUPPLIED

Reagents are analytical grade and are compatible with protease & phosphatase inhibitors. **All kit components can be stored between 2 °C and room temperature.**

- **Dynabeads[®] M-270 Epoxy** (> 60 mg)
- **C1** (20 ml)
- **C2** (8 ml)
- **SB** (40 ml)
- **LB** (15 ml)
- **HB** (15 ml)

1.4 ADDITIONAL MATERIALS REQUIRED

- Magnet: e.g. DynaMag[™]-2 (see www.invitrogen.com/magnets).
- Mixer allowing rotation or tilting of tubes.
- Antibodies or other proteins to be coupled (of your choice).

2. ANTIBODY COUPLING CONSIDERATIONS

2.1 OPTIMAL ANTIBODY USE

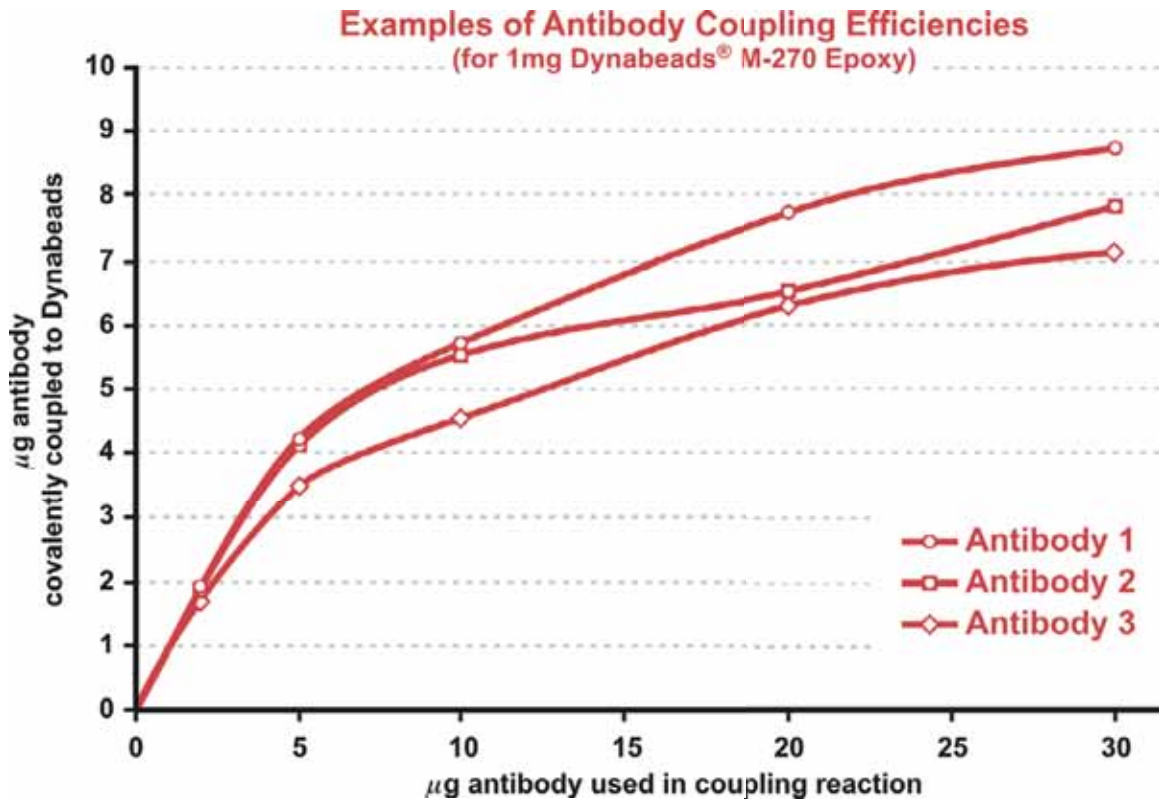
Antibody coupling to Dynabeads is most efficient when using low quantities of antibody per mg beads (5-10 µg antibody per mg of beads, see **Figure 1**). This same general rule applies when coupling other proteins (e.g. lectins, enzymes, etc.) although the “optimal coupling range” may differ for different ligands.

For some assays, saturation of the Dynabeads with antibodies or other proteins (hereafter referred to collectively as “ligand”) may be desirable. In such cases the excess coupled ligand may provide an increased blocking effect on the bead surface, potentially reducing non-specific binding even further. Using excess ligand in the coupling reaction will significantly increase the ligand consumption, along with the potential for (non-covalently) adsorbed ligand to “leak” off the beads.

To help prevent any “leakage” of non-coupled (non-covalently bound) ligand in the downstream assay, a couple of additional bead washing steps can be performed at the end of the coupling protocol. These washes will help to remove non-coupled ligands from the beads using a buffer appropriate to your ligand (e.g. PBST).

Note: We recommend including 0.01% - 0.1% Tween in wash buffers.

FIGURE 1



2.2 ANTIBODY (OR LIGAND) SELECTION

The choice of antibody (or other protein, e.g. lectin, enzyme, etc.) is the most important factor for successful target capture. **Note that not all antibodies are suitable for all applications.** While a particular antibody (or lectin, enzyme, etc.) may recognize and bind to (or cleave, phosphorylate, etc.) its target in some applications (e.g. Western Blotting), there is no guarantee that the same antibody, will function well in an immunoassay or in immunoprecipitation or co-immunoprecipitation. Please refer to the manufacturer's recommendations regarding your ligand.

2.3 ANTIBODY ADDITIVES

- **SODIUM AZIDE (NaN₃)**

Many commercially available antibodies contain NaN₃ as preservative. The presence of NaN₃ can lead to a small decrease (<10%) in antibody coupling efficiency. This will not be a problem for most applications. Furthermore, if desired this can be easily compensated by slightly increasing the quantity of antibody used in the coupling reaction. Alternatively the NaN₃ can be removed prior to coupling by standard gel filtration chromatography or dialysis.

- **ANTIBODY STABILIZING PROTEINS**

Some commercially available antibodies (proteins and enzymes) contain protein additives such as BSA or Gelatin. Protein additives present during the coupling reaction will be coupled to the bead surface along with antibody (or other input protein). The presence of protein additives will not affect antibody coupling efficiency if the total quantity of protein (antibody + protein additive) in the coupling reaction does not exceed the capacity of the beads. In the

case of BSA or Gelatin, the coupled proteins may provide a beneficial blocking effect but may also result in the co-isolation of BSA or Gelatin interacting proteins.

- **GLYCEROL**

Coupling of antibodies or other proteins stabilized in glycerol is not recommended. Although it is possible to couple such ligands, the antibody (or protein) function may be severely affected.

2.4 FUNCTION AND STABILITY OF ANTIBODIES & LIGANDS COUPLED TO DYNABEADS

Different antibodies, proteins, enzymes, etc. have different characteristics. Even different antibody clones raised in the same species against the same antigen can vary greatly in pI, antigen binding affinity, and stability. Consequently the coupling efficiency will vary slightly between different batches of antibodies, proteins & enzymes. Furthermore, some coupled ligands will retain their function for months, even years when stored properly, while others will lose their function within several weeks. These are entirely ligand dependent.

2.5 ANTIBODY QUANTITY

The quantity of Dynabeads used for coupling will depend upon the number and scale of the downstream assays to be run. Antibody (or other protein ligand) coupling reactions should be scaled as outlined in **TABLE 1**. For best immunoprecipitation and co-immunoprecipitation results, coupling of 5 µg to 7 µg antibodies per mg Dynabeads is recommended for high affinity antibodies. For best results in immunoassays or other downstream assays involving the coupling of antibodies or lectins or other proteins to Dynabeads, the optimal µg of input ligand may vary according to the ligand and the assay.

Coupling of more antibodies per mg Dynabeads may be necessary if the antibody affinity is poor. Typically, for immunoprecipitation and co-immunoprecipitation of protein complexes with analysis by silver staining or Western blotting, 1.5 mg of antibody-coupled beads are used. For the detection by Coomassie staining, 7.5 mg of antibody coupled beads are used.

If ligand cost is not a factor, we recommend that you begin by using 20 µg – 30 µg ligand per milligram Dynabeads[®] M-270 Epoxy.

2.6 ANTIBODY AGGREGATES AND ANTIBODY LEAKAGE

The presence of antibody aggregates in the antibody stock used for coupling can result in antibody leakage during the downstream assay. To help reduce this we recommend that antibody aggregates be removed from the antibody stock by centrifugation at 16,000 × *g* for 10 min at 4°C.

3. ANTIBODY COUPLING PROTOCOL

Note: the following protocol may be used for coupling antibodies or other proteins (such as lectins, functional enzymes, etc.) to the included Dynabeads® M-270 Epoxy.

Day 1

- 1) **WEIGH** out the appropriate amount of Dynabeads® M-270 Epoxy (according to **Table 1**).

Moisture on unused beads will deactivate the reactive groups necessary for covalent antibody coupling. **To avoid condensation on unused beads, make sure the beads are at room temperature prior to opening the bottle.**

TABLE 1: CALCULATION OF ANTIBODY AND C1 VOLUMES

Beads (mg)	Volumes in μ l			Total Reaction Volume
	Antibody	C1	C2	
5	V	250 - V	250	500
10	W	500 - W	500	1000
20	X	1000 - X	1000	2000
40	Y	2000 - Y	2000	4000
60	Z	3000 - Z	3000	6000

Rule of Thumb: The total reaction volume (C1 - μ l Ab + C2) should be 100 μ l per mg beads. The C1 + Ab volume is equal to C2 volume.

- 2) **WASH** the beads: Add 1 ml of **C1** to the beads and mix by vortexing or pipetting.
- 3) **PLACE** the tube on a magnet and allow the beads to collect at the tube wall. Remove the supernatant.

- 4) **ADD** the appropriate volume of antibody + **C1** (according to **Table 1**) to the washed beads and mix by vortexing or pipetting.

Example: If you are coupling 5 mg Dynabeads and your required quantity of antibodies has a volume of 100 µl, you will need to add 150 µl of C1 (i.e. 250µl C1 – 100µl Ab = 150µl.)

- 5) **ADD** the appropriate volume of **C2** and mix by vortexing or pipetting.
- 6) **INCUBATE** on a roller at 37°C overnight (16-24 hours). Make sure the fluid in the tube is mixing well.

Make sure the beads do not settle. Beads settling during the overnight incubation will result in inefficient antibody coupling.

Day 2

- 7) **PLACE** the tube on a magnet. Allow the beads to collect at the tube wall. Remove the supernatant.

TABLE 2: REQUIRED BUFFER VOLUMES

Beads (mg)	Volumes in µl			
	HB	LB	SB	SB (for storage)
5	800	800	800	500
10	800	800	800	1000
20	1600	1600	1600	2000
40	1600	1600	1600	4000
60	1600	1600	1600	6000

- 8) **HB WASH:** Add 0.8 or 1.6 ml (**Table 2**) of **HB** and mix by vortexing or pipetting. Place the tube on a magnet, allow the beads to collect at the tube wall, then remove the supernatant.
- 9) **LB WASH:** Add 0.8 or 1.6 ml (**Table 2**) of **LB** and mix by vortexing or pipetting. Place the tube on a magnet, allow the beads to collect at the tube wall. Remove the supernatant.
- 10) **SHORT SB WASH:** Add 0.8 or 1.6 ml (**Table 2**) of **SB** and mix by vortexing or pipetting. Place the tube on a magnet, allow the beads to collect at the tube wall, then remove the supernatant. Repeat the wash once more.
- 11) **LONG SB WASH:** Add 0.8 or 1.6 ml (**Table 2**) of **SB** and mix by vortexing or pipetting. Incubate on a roller/rotator at RT for 15 minutes. Place the tube on a magnet, allow the beads to collect at the tube wall, then remove the supernatant.
- 12) **RESUSPEND** beads in the same volume of **SB** as was the total coupling reaction volume and store at 4°C until use. The final bead concentration is **10 mg antibody coupled beads/ml**. ***Your beads are now covalently coupled with antibody and ready for IP, Co-IP, a her assays.***

If desired, antibody coupled beads may be concentrated up to 30 mg/ml by reducing the storage buffer volume.

For long term storage, a final concentration of 0.02% NaN₃ may be added to the antibody coupled beads.

Warning: Not all coupled antibodies retain their function in long term storage. Verify your coupled antibody stability by testing in small scale.

4. GENERAL INFORMATION

Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

4.1 Precautions

These products are designed to be used with very strong permanent magnets. People wearing a pacemaker or any other medical magnetizable implant should not use this product unless advised by a health professional; the implant could be affected or damaged by exposure to a strong magnetic field.

Keep magnetizable tools and objects out of the working area. This includes, but is not restricted to, credit cards and other products containing magnetic recording devices. Keep away from delicate instruments, watches, electronic equipment, displays and monitors.

Magnets may attract steel or other magnetic material with high mechanical forces. Take care during handling. Avoid contact between two magnets. Do not pull the magnets apart if contact has been made; twist off to prevent damage to the unit or fingers. The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to. IN NO EVENT SHALL INVITROGEN DYNAL AS BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES.

4.2 Disinfection of DynaMag™ Magnets

The following materials have been tested for cleaning purposes. Spray and/or wipe the DynaMag unit with one of the following cleaning agents:

- 70% isopropyl alcohol
- 1% sodium hypochlorite solution (Bleach)
- 0.1N HCl solution

Other disinfectants have not been tested and may not be suitable. Do not submerge in aqueous solutions and avoid prolonged exposure to water or aqueous solutions.

Clean with a damp cloth and mild detergent when exposed to harsh solvents. Do not autoclave the DynaMag magnets.

4.3 Storage and Stability

All kit components can be stored between 2°C and room temperature.

Precautions should be taken to prevent bacterial contamination of the beads.

When stored in unopened vials at 2°C – Room Temperature, the Dynabeads and buffers provided in this kit are stable until the expiration date printed on the label.

Beads should not be autoclaved, but can be incubated with ethanol (70%, 1 hour) or gamma irradiated.

Coated beads may be stored at 2-8°C for several weeks or even months, depending on the stability of the immobilized ligand. Coated beads should be washed once for 5 min in PBS/BSA before use. Use the magnet to collect the beads according to the washing procedure.

If a preservative is needed for storage of coated beads, a final concentration of 0.02% (w/v) sodium azide (NaN_3) may be added to the storage buffer. Carefully remove before use by washing (see 3.2 above). Required safety precautions must be followed when handling this cytotoxic material.

4.4 Technical Support

Please contact Invitrogen Dynal® for further technical information (see contact details).

4.5 Warning and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Sodium azide is toxic if ingested. Avoid pipetting by mouth. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide buildup.

This product guarantees optimum isolation of Dynabeads, not the isolation of a specific material. Recovery of biomolecules by magnetic isolation depends on the avidity of the antibodies or ligands on the surface of Dynabeads, as well as factors concerning the biomolecules themselves and the matrix from which they are to be isolated. Material Safety Data Sheet (MSDS) is available at <http://www.invitrogen.com>.

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1600 Faraday Avenue, Carlsbad, California 92008.

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Fax (760) 602-6500.

Email: outlicensing@invitrogen.com

4.9 Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

This warranty shall not apply to any products which shall have been altered outside Invitrogen Dynal, nor shall it apply to any products which have been subjected to misuse or mishandling.

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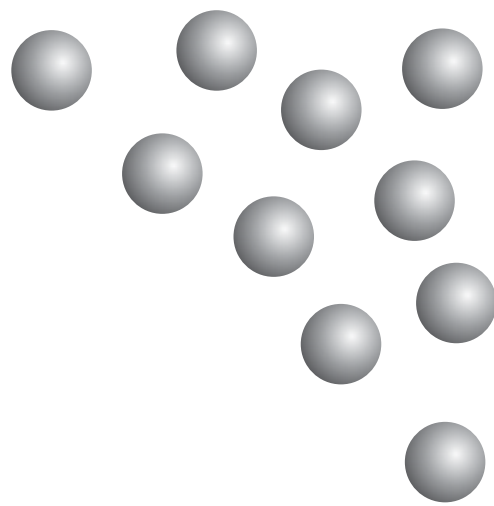
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