USER GUIDE

novex® by life technologies™

Dynabeads® Antibody Coupling Kit

Catalog number 14311D

Revision Date June 2012 (Rev. 001)

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

life technologies™

Contents

Kit Contents and Storage

Storage

Upon receipt, store all components of the Dynabeads® Antibody Coupling Kit at room temperature.

When stored in unopened vials at 2°C to room temperature, the Dynabeads® M-270 Epoxy and buffers provided in this kit are stable until the expiration date printed on the label.

Kit contents

The components included in the Dynabeads® Antibody Coupling Kit are listed in the following table.

Component	Amount
Dynabeads® M-270 Epoxy	>60 mg
C1	20 mL
C2	8 mL
HB	15 mL
LB	15 mL
SB	40 mL

Product Use

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

Description of the System

About the Kit

System overview

The Dynabeads® Antibody Coupling Kit enables covalent coupling of antibodies (or other proteins such as lectins, functional enzymes, hereafter collectively referred to as "ligand") of your choice onto the surface of Dynabeads® M-270 Epoxy. Once the coupling reaction is complete, the resulting Dynabeads® M-270 surface exhibits ultra-low background binding. It is therefore not necessary to block the bead surface prior to use.

Following immobilization of your ligand of choice on the beads, the ligand-coupled beads may then be used for experiments such as immunoassays, immune-precipitation (IP), co-immunoprecipitation (Co-IP) of protein complexes, Co-IP of protein-nucleic acid complexes, as well as many other downstream applications. Downstream assays could include those requiring ligands such as lectins, enzymes, or others to be coupled to the Dynabeads® M-270 Epoxy instead of antibodies.

Captured proteins, protein complexes, and protein-nucleic acid complexes are easily separated from lysate using the magnetic properties of Dynabeads® M-270 Epoxy in combination with a DynaMag™ magnet facilitating washing, buffer changes, and elution.

Continued on next page

Advantages of the Dynabeads® Antibody Coupling Kit

- Highly efficient covalent binding of most common antibody species to Dynabeads® M-270 Epoxy.
- Antibody/Ligand-coupled Dynabeads® M-270 Epoxy exhibit ultra-low background binding eliminating the need for blocking.
- Magnetic separation facilitates washing, buffer changes, and elution.
- Other protein ligands (e.g., lectins, enzymes, etc.) can be covalently coupled to the surface of Dynabeads® M-270 Epoxy using the same beads, buffers and protocol provided in this kit.

Coupling Considerations

Antibody or Ligand Selection

The choice of antibody or ligand is the most important factor for successful target capture.

Note: Not all antibodies are suitable for all applications

While a particular antibody (or lectin, enzyme) 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, IP, or Co-IP. Refer to the manufacturer's recommendations regarding your antibody/ligand.

Dynabeads® M-270 Epoxy Coupling Guidelines

- Use low quantities of ligand per mg of beads
- Optimal coupling is achieved using purified ligand
- Coupling of antibodies or other proteins stabilized in glycerol is not recommended

Additives

Common additives in antibody preparations which may affect coupling to Dynabeads® (see **Antibody Additives**, page **11**, for a more detailed discussion) include:

- Sodium Azide (NaN_3)
- Antibody Stabilizing Proteins
  - Bovine Serum Albumin (BSA)
  - Gelatin
- Glycerol

Continued on next page

Coupling Considerations, continued

Ligand Use

- Optimal coupling at 5–10 µg ligand per mg of beads (see **Ligand Binding Curve** page **10**). Low affinity antibodies may require increasing the amount of input antibody.
- "Optimal coupling range" is ligand dependent and determined empirically.
- Using excess ligand significantly increases ligand consumption. If ligand cost is not a factor, we recommend using 20–30 µg ligand per mg Dynabeads® M-270 Epoxy.
- Dynabeads® M-270 saturation binding may be desirable. Excess coupled ligand may potentially reduce non-specific binding even further.
- Using excess ligand increases the potential for "leakage" of non-covalently adsorbed ligand in downstream assays. Additional bead washing steps after coupling will reduce "leakage".

Ligand Dependent Variability

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, and enzymes. Furthermore, some coupled ligands retain their function for months or even years when stored properly, while others lose their function within several weeks. These are entirely ligand dependent.

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 removing the antibody aggregates from the antibody stock by centrifugation at 16,000 × *g* for 10 min at 4ºC.

Coupling Protocol

Introduction

The following protocol may be used for coupling antibodies or other ligands (such as lectins, functional enzymes) to the included Dynabeads® M-270 Epoxy. The quantity of Dynabeads® M-270 Epoxy used for coupling depends upon the number and scale of the downstream assays to be run.

Required materials

- Magnet: e.g., DynaMag™-2 (see **www.lifetechnologies.com/magnets**).
- Mixer allowing rotation or tilting of tubes.
- Antibodies or other protein ligands to be coupled (of your choice).
- Centrifuge capable of achieving >16,000 × *g*
- Phosphate Buffered Saline with 0.01–0.1% Tween®-20 (PBST)
- Bovine Serum Albumin (BSA)

Scale of Coupling Reaction

Antibody (or other protein ligand) coupling reactions should be scaled as outlined in **Calculation of Antibody and C1 Volumes** (page **6**). Typically, for analysis by silver staining or western blotting, 1.5 mg of antibody-coupled beads are used per IP or Co-IP of protein complexes. For detection by Coomassie staining, 7.5 mg of antibody coupled beads are used per IP or Co-IP reaction.

Continued on next page

Antibody Coupling Protocol (Day 1)

Important: Moisture on unused beads deactivates the reactive groups necessary for covalent antibody coupling. To avoid condensation on unused beads, ensure the beads are at room temperature prior to opening the bottle.

1. Disinfect the magnet you will be using to prevent accidental sample contamination.
2. Weigh out the appropriate amount of Dynabeads® M-270 Epoxy (see **Calculation of Antibody and C1 Volumes** in the following table).
3. Wash the beads with 1 mL of **C1** and mix by vortexing or pipetting.
4. Place the tube on a magnet for 1 minute and allow the beads to collect at the tube wall. Remove the supernatant.
5. Add the appropriate volume of antibody + **C1** (see **Calculation of Antibody and C1 Volumes** in the following table) to the washed beads and mix by gentle vortexing or pipetting.

   Example: If you are coupling 5 mg Dynabeads® and your required quantity of antibody has a volume of 100 µL, you need to add 150 µL of **C1** (i.e., 250 µL **C1** – 100 µL Ab = 150 µL.)
6. Add the appropriate volume of **C2** and mix by gentle vortexing or pipetting.

Calculation of Antibody and C1 Volumes

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

	Volume (µL)		
Beads (mg)	**C1**	**C2**	**Total Volume**
5	250 – vol. Ab	250	500
20	1000 – vol. Ab	1000	2000
60	3000 – vol. Ab	3000	6000

Continued on next page

Antibody Coupling Protocol (Day 1), continued

7. Incubate on a roller at 37°C overnight (16–24 hours). Make sure the fluid in the tube is mixing well.

   Important: Make sure the beads do not settle, because this will result in inefficient antibody coupling.

Antibody Coupling Protocol (Day 2)

Note: We recommend including 0.01%–0.1% Tween®20 in **HB** and **LB** wash buffers for improved stringency.

1. Place the tube on a magnet for 1 minute and allow the beads to collect at the tube wall. Remove the supernatant.
2. **HB** wash: Add the appropriate volume of **HB** and mix by vortexing or pipetting.

Beads (mg)	Volume HB (µL)
<20	800
≥20	1600

3. Place the tube on a magnet for 1 minute and allow the beads to collect at the tube wall. Remove the supernatant.
4. **LB** wash: Add the appropriate volume of **LB** and mix by vortexing or pipetting.

Beads (mg)	Volume LB (µL)
<20	800
≥20	1600

5. Place the tube on a magnet for 1 minute and allow the beads to collect at the tube wall. Remove the supernatant.

Continued on next page

Antibody Coupling Protocol (Day 2), continued

6. Short **SB** wash: Add the appropriate volume of **SB** and mix by vortexing or pipetting.

Beads (mg)	Volume SB (µL)
<20	800
≥20	1600

7. Place the tube on a magnet for 1 minute and allow the beads to collect at the tube wall. Remove the supernatant.
8. Repeat **Short SB** wash once more.
   Note: If antibody leakage is determined to be a problem repeat this step one or two more times.
9. Long **SB** Wash: Add the appropriate volume of **SB** and mix by vortexing or pipetting.

Beads (mg)	Volume SB (µL)
<20	800
≥20	1600

10. Incubate on a roller/rotator at room temperature for 15 minutes.
11. Place the tube on a magnet for 1 minute and allow the beads to collect at the tube wall. Remove the supernatant.
12. Resuspend antibody-coupled beads in 100 µL **SB** per mg beads and store at 2°C to 8°C until use. The final bead concentration is 10 mg/mL antibody-coupled beads.
13. If desired, antibody-coupled beads may be concentrated up to 30 mg/mL by reducing the storage buffer volume. Your beads are now covalently coupled with antibody and ready for IP, Co-IP, or other assays.

Continued on next page

Long Term Storage

Coated beads may be stored at 2°C to 8°C for several weeks or even months, depending on the stability of the immobilized antibody/ligand. If a preservative is needed for long term storage of coated beads, a final concentration of 0.02% (w/v) sodium azide may be added to the storage buffer. Wash coated beads once for 5 minutes in PBS with 0.1% BSA before use.

CAUTION

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Important

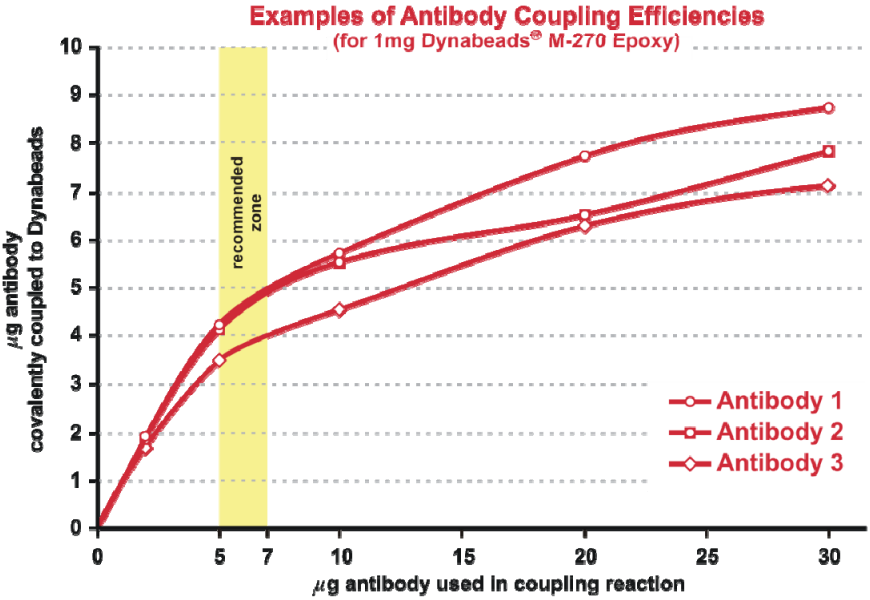
Not all coupled antibodies retain their function in long term storage. Verify your coupled antibody stability by testing in small scale experiment.

Appendix

Dynabeads® Binding Capacity

Ligand Binding Curve

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



Antibody Additives

Sodium Azide

Many commercially available antibodies contain sodium azide as preservative. The presence of sodium azide 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 sodium azide 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
- 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 negatively affected.

Accessory Products

Related Products

The following products may be used with the Dynabeads® Antibody Coupling Kit. For details, visit **www.lifetechnologies.com** or contact **Technical Support** (see page **13**).

Product	Quantity	Cat. no.
DynaMag™-2	Each	12321D
SampleRack (for DynaMag™-2)	Each	12322D
Dynabeads® Co-Immunoprecipitation Kit	1 Kit	14321D
Dynabeads® M-270 Epoxy	60 mg	14301
Dynabeads® M-270 Amine	2 mL	14307D

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetechnologies.com**)
- Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis is available at **www.lifetechnologies.com/support**. Search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

General Information

Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

SPEC-06949

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit
www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™