

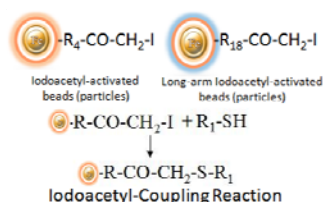
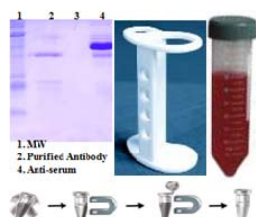
EcoMag™ Quick Peptide Conjugation Kit

Introduction

EcoMag™ Quick Peptide Conjugation Kit is specifically designed for quick and efficient conjugation of peptides to Long-arm Iodoacetyl-activated magnetic particles for anti-antigen peptide antibody purification from serum of various animal species.

Features and benefits:

1. Covalently and specifically couples with cysteine residues
2. Water-insoluble ligands can be conjugated in coupling buffer containing final concentration of 10-30% acetonitrile, or dimethylformamide (DMF), or dimethylsulfoxide (DMSO) or In coupling buffer containing final concentration of 6 M Guanidine•HCl.
3. Stable covalent bond with low levels of ligand leakage
4. Produces reusable immunoaffinity matrices
5. Low nonspecific binding
6. No filtration during antibody purification from serum
7. Quick procedure (30-60 minutes offhand coupling, 20 minutes antibody purification)
8. Immobilize 1-10 mg protein or 0.1-1 mg peptide/ml Particles
9. Application: Purification for Antibody, Protein/Peptide



Cat#	Description
MF-101	EcoMag™ Quick Peptide Conjugation Kit Kit Components EcoMag Long-arm Iodoacetyl-activated Magnetic Particles 500 mg (Store at -20°C protected from light and free of moisture upon receipt) 10x Conjugation Buffer: 12 ml coupling Buffer (0.5 M Tris, 50 mM EDTA-Na, pH 8.5) 5x Wash Buffer: 24 ml (5 M NaCl, 0.05% NaN3)
MF-102	EcoMag™ Quick Peptide Conjugation Kit Kit Components EcoMag Long-arm Iodoacetyl-activated Magnetic Particles 2.5 g (Store at -20°C protected from light and free of moisture upon receipt) 10x Conjugation Buffer: 60 ml coupling Buffer (0.5 M Tris, 50 mM EDTA-Na, pH 8.5) 5x Wash Buffer: 120 ml (5 M NaCl, 0.05% NaN3)
MF-103	EcoMag™ Quick Peptide Conjugation Kit Kit Components EcoMag™ Long-arm Iodoacetyl-activated Magnetic Particles 25 g (Store at -20°C protected from light and free of moisture upon receipt) 10x Conjugation Buffer: 3x 200 ml coupling Buffer (0.5 M Tris, 50 mM EDTA-Na, pH 8.5) 5x Wash Buffer: 5x 250 ml (5 M NaCl, 0.05% NaN3)
MF-104	EcoMag™ Quick Peptide Conjugation Kit Kit Components EcoMag Long-arm Iodoacetyl-activated Magnetic Particles 50 g (Store at -20°C protected from light and free of moisture upon receipt) 10x Conjugation Buffer: 6x 200 ml coupling Buffer (0.5 M Tris, 50 mM EDTA-Na, pH 8.5) 5x Wash Buffer: 10x 250 ml (5 M NaCl, 0.05% NaN3)

*Store at 4°C protected from light and free of moisture upon receipt

Protocol

Note:

- The following protocol is an example for coupling protein and/or peptides to EcoMag™ Long-arm Iodoacetyl-Activated magnetic Particles. It is strongly recommended that a titration be performed to optimize the quantity of Particles used for each individual application. This protocol can be scaled up and down accordingly.

Materials Required

▪ Coupling Buffer

- 1) Soluble coupling buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5
- 2) Insoluble coupling buffer: 50 mM Tris, 5 mM EDTA-Na, pH 8.5, 20- 30% DMSO or DMF or 6 M Guanidine•HCl



- **Wash Buffer:** 1 M sodium chloride (NaCl) in distilled H₂O
- **Phosphate buffered saline (PBS)**
- **Magnetic Separator (for manual operation):** Based on sample volume, user can choose one of the following magnetic Separators: BcMag separator-2 for holding two individual 1.5 ml centrifuge tubes (Cat. # MS-01); BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes (Cat. # MS-02); BcMag separator-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Cat. # MS-03); BcMag separator-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Cat. # MS-04); BcMag™ separator-96 for holding a 96 ELISA plate (Cat.# MS-05).

A. Sample Preparation

Note:

- *Make sure that peptide to be conjugated has free (reduced) sulfhydryls. To ensure free sulfhydryl groups available, disulfide bonds must be reduced with a reducing agent such as DTT (dithiothreitol), TCEP (tris(2-carboxyethyl)phosphine), or 2-MEA (2-Mercaptoethylamine•HCl) followed by desalting or dialysis to remove the reducing agent.*
 - *Newly Synthesized peptides may be directly used for coupling, if used immediately after reconstitution*
 - *If the sample contains reducing agents with free sulfhydryls (e.g., 2-mercaptoethanol, DTT or TCEP), these agents must be completely removed by dialysis or desalting.*
1. Dissolve 1mg peptide in 1ml soluble coupling buffer if soluble. If insoluble, dissolve in 1ml Insoluble coupling buffer
 2. If samples have already suspended in other buffer, dilute samples with equal volume of coupling buffer.

B. Magnetic Particles Preparation

1. Weight and transfer 50 mg Magnetic Particles to a centrifuge tube. Resuspend the Particles by adding 1 ml coupling buffer and mix the Particles by vigorous vortexing for 1-2 minutes.

Note:

- *Once rehydrated, the Particles should be used as soon as possible due to stability of functional group.*
2. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator. Remove the tube from the separator and resuspend the Particles with 1 ml coupling buffer by vortex for 30 seconds.
 3. Repeat step-2 once.

C. Coupling

1. Add sample from A1 or A2 to the washed magnetic Particles and incubate for 30-60 minutes at room temperature with gentle rotation.
2. Washed the magnetic Particles with 1ml Coupling buffer for four times as described in B2.
3. Resuspend the Particles in PBS buffer containing 0.05% sodium azide and store at 4°C.

Antibody Purification

Buffer Composition

- Binding/Washing Buffer (57.7 mM Na₂HPO₄, 42.3 mM NaH₂PO₄, pH 7.0)
- Elution Buffer (0.2 M Glycine/HCl, pH 2.5)
- Neutralization Buffer (1.0 M Tris-HCl, pH 9.0)

Protocol

The protocol can be properly scaled up or down.

Note:

1. This protocol is optimized for purifying most IgG antibodies from different sources. However, it is impossible to design a universal kit for all IgG purification because no two antibodies are exactly alike. In order to obtain the best results, each user must determine the optimal working conditions for purification of individual antibodies, especially for those weakly-binding antibodies, based on suggestions in the Troubleshooting section.
2. To ensure optimal binding conditions involving ionic strength and pH, it is necessary to dilute serum samples, ascites fluid or tissue culture at least 1:1 with Binding/Washing buffer prior to the purification. Remove any insoluble materials in the sample by centrifugation or filtration through a 0.2 µm filter.
3. Prior to purifying IgG, the user should equilibrate all the reagents contained in the kit to room temperature and make 1x working solutions by diluting 5x stock solutions with 4 volumes of double distilled H₂O.



A. Purification

1. Gently shake the bottle containing peptide-conjugated Particles until the magnetic Particles are completely suspended. Transfer an appropriate amount of the Particles to a fresh tube.

Note: *The optimal amount of Particles to be used should be empirically determined by each user based on the amount of the IgG in the crude sample. Too many magnetic Particles will result in a higher background; too little will reduce the yield. We recommend 100 μ l of the completely suspended Particles per 100 μ g of IgG antibodies. Usually a high-titer rabbit antiserum has roughly 5 mg/ml of IgG, Mouse ascites has roughly 10 mg/ml of IgG, and goat or sheep antiserum has roughly 20 mg/ml of IgG.*

2. Place the tube on a magnetic separator for 1 minute. Remove the supernatant while the tube remains on the separator. Remove the tube from the separator and wash the Particles with 5 Particles volumes of 1x Binding/Washing Buffer by vortex. Place the tube on the magnetic separator for 1 minute. Remove the supernatant while the tube remains on the separator.
3. Repeat step 2 twice.
4. Remove the tube from the magnetic separator and re-suspend the Particles by adding appropriate amount of antibody sample/Binding Buffer solution (Mix crude or diluted antibody sample with 1x Binding/Washing Buffer at ratio of 1:2). Mix well by gently pipetting several times and incubate at room temperature for 10-20 minutes or 4° C for 30-45 minutes with rotational mixing.
5. Wash the Particles with Binding/Washing buffer as in step 2 until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).
6. Add an appropriate amount of Elution Buffer to elute the IgG from the magnetic Particles. Mix well by gently pipetting several times and incubate at 4° C for 10 minutes with rotational mixing. Place the tube in the magnetic separator for 1 minute and carefully remove the antibody-containing supernatant into a clean tube. Immediately neutralize the eluted antibody solution by adding 0.1 ml neutralization buffer for each 1.0 ml supernatant and mix well.
7. Desalt and concentrate the eluted fraction by dialysis, gel filtration chromatography or other methods.

General Affinity Purification Protocol

1. Transfer optimal amount of the Particles to a centrifuge tube. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.

Note:

- *It is strongly recommended that a titration be performed to optimize the quantity of Particles used for each individual application based on the amount of the target protein in crude sample. Too many magnetic Particles used will cause higher backgrounds, while too little Particles used will cause lower yields. Each mg of conjugated magnetic Particles normally bind to 1-20 μ g target protein.*
2. Remove the tube and resuspend the Particles with 5 bed Particles volume of PBS buffer by vortex for 30 seconds. Leave the tube at room temperature for 1-3 minutes. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
 3. Repeat step 2 two times
 4. Add washed Particles to crude sample containing target protein and incubate at room temperature or desired temperature for 1-2 hours (Lower temperature require longer incubation time).
 5. Extensively wash the Particles with 5 bed Particles volumes of PBS buffer or 1M NaCl until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).
 6. Elute the target protein by appropriated methods such as low pH (2-4), high pH (10-12), high salt, high temperature , affinity elution or boiling in SDS-PAGE loading buffer.