

## Rapid Removal of anti-*E. coli* Protein Antibodies Kit

### Introduction

The Rapid Removal of anti-*E. coli* Protein Antibodies kit is designed for rapid, single-step removal of contaminating *E. coli*-reactive antibodies from antibody solutions based on magnetic beads.

### Components

Contents	Cat. No.	ME 101	ME 102	ME 103	ME 104	ME 105	ME 106
BcMag. <i>E. coli</i> Protein Magnetic Beads		2.0ml	5.0ml	10ml	2.0ml	5.0ml	10ml
10x <i>E. coli</i> Protein Equilibration Buffer		2.0ml	5.0ml	10ml	X	X	X
5x <i>E. coli</i> Protein Regeneration Buffer		2.0ml	5.0ml	10ml	X	X	X

### Buffer Composition

- BcMag.*E. coli* Protein Magnetic Beads (Suspended in 10 mM Tris, 0.15 M NaCl, 0.1% BSA, 1 mM EDTA, pH 7.4, 0.1% NaN<sub>3</sub>)
- 1x *E. coli* Protein Equilibration Buffer (0.05 M Phosphate Buffer, pH 7.5, containing 0.15 M NaCl)
- 1x *E. coli* Protein Regeneration Buffer (0.1M Glycine, pH 2.8)

**Binding capacity:** 10 mg/ml magnetic beads

**Storage:** 4° C (Do not freeze)

### Protocol

**Note:** Prior to purifying antibodies, you should equilibrate all the reagents contained in the kit to room temperature .

#### A. Removal of contaminating *E. coli*-reactive antibodies

1. Gently shake the bottle of *E. coli* Protein-Magnetic Beads until the magnetic beads are completely suspended. Transfer the desired amount of beads to a fresh tube.

**Note:** Use 100 µl of the magnetic beads per 1.0 mg antibody.

2. Place the tube in a magnetic separator and wait for 1 min until the supernatant becomes clear. Completely remove and discard the supernatant. Suspend the beads with 4 volumes of 1x Equilibration Buffer. Mix well by pipetting several times. Place the tube in the magnetic separator, wait for 1 min and discard the supernatant.

**Note:** Magnetic separators are commercially available from Bioclone Inc.: BcMag separator-2 for holding two individual 1.5 ml centrifuge tubes, Cat. No. MS-01; BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes, Cat. No. MS-02; BcMag separator-24 for holding twenty-four individual 1.5 ml centrifuge tubes, Cat. No. MS-03; BcMag separator-50 for holding one 50 ml and one 15 ml centrifuge tube, Cat. No. MS-04.

3. Repeat (step 2) one time.

4. Add appropriate amount of antibody-containing sample, remove the tube from the magnetic separator, mix the beads very well by pipetting several times and incubate at room

temperature for 20 min with rotational mixing.

5. Place the tube in the magnetic separator, wait for 3 min and carefully transfer the antibody-containing supernatant into a fresh tube and the purified antibody is ready for desired use.

#### B. Beads regeneration and storage

1. Immediately wash the beads after step 5 above by adding 4 volumes of 1x Regeneration Buffer and mix well by pipetting several times.

2. Place the tube in the magnetic separator, wait for 1 min and discard the supernatant.

3. Re-suspend the magnetic beads by adding 0.5 volumes of 1x Equilibration Buffer and 0.02% sodium azide.

4. Store at 4° C

**Note:** The regenerated magnetic beads can only be used with the same antibody due to the risk of cross-contamination with a different antibody.