

Human EGFR-coupled Magnetic Beads

Cat.No. MBE-K012

■ Product Description

The Human EGFR-coupled Magnetic Beads are superparamagnetic beads conjugated with streptavidin, which is then coupled with biotinylated human EGFR protein. This takes advantage of the extraordinarily strong interaction between biotin and streptavidin, meaning the protein is effectively bound irreversibly. These characters enable the beads to achieve a high yield and low non-specific binding in protein separation.

These beads can be utilized for a wide array of applications, including biopanning, immunocapture, flow cytometry, etc.. This product offers a convenient and efficient way to capture proteins of interest in solution, all you need is a magnet.

■ Protein Information

Protein Description: Biotinylated Human EGF R Protein, His,Avitag™ (MALS verified)

Expression platform: HEK293 cells

AA range: AA Leu 25 - Ser 645 (Accession # P00533-1)

Calculated MW: 72.3 kDa

■ Beads Information

Bead size: 2 mg/5 mg

Particle size: 2 μm

Magnetism: Superparamagnetic

Number of beads/mg: 1X10⁸ beads/mg

Amount of protein coupled: 484 pmol (35 μg) EGFR/mg Beads

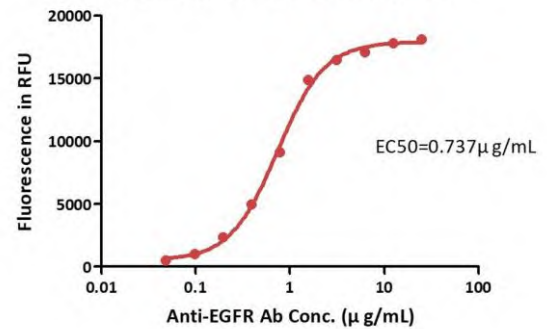
■ Storage

This product is stable for 1 year when stored at -20 °C.

Please avoid more than 3 freeze-thaw cycles once reconstitution, immediate use after reconstitution is highly recommended.

■ Sample Data

Anti-EGFR Ab binding with EGFR pre-coupled beads
35 μg/mL EGFR Coupled onto 1mg Beads



Immobilized 35 μg EGFR protein/1 mg beads (1 mg/mL) can bind the Anti-EGFR Antibody (Cetuximab) with an EC50 of 0.737 μg/mL. PE-labeled anti-human IgG Fc antibody was used for detection (QC tested).

■ General guidelines

Use a magnetic separator that is suitable for your equipment and application. Allow the beads to separate for at least 1 minute before removing supernatant. The beads are dense and will tend to settle very quickly. Be sure that any bead mixture is homogenous before aliquoting.

Before any experiment, it is necessary to wash the magnetic beads and remove the trehalose from the formulation buffer and change the buffer to your Assay/Washing Buffer.

1. Place the tube with reconstituted beads on a magnetic separator for 2 min. Remove the supernatant.
2. Remove the tube from the magnetic separator and resuspend the pelleted beads in equal volume (or at least 1000μL) of Assay/Washing Buffer. Mix by vortex for approximately 10 sec.
3. Place the tube on the magnetic separator for 2 min. Remove the supernatant.
4. Wash the beads for three times in total by repeating steps 2 and 3.

■ Binding assay

Below is a suggested antibody capture protocol for your reference.

1. Reconstitute and wash the magnetic beads according to the Certificate of Analysis. Immediate use is strongly recommended.
2. Add 1mL Assay/Washing Buffer per mg Beads to resuspend the beads. The most common Assay/Washing Buffer is PBS, pH 7.3, with 0.05% Tween-20. Optionally, you may add 0.05% BSA. When assaying a serum sample, please choose a Special Assay/Blocking Buffer (Cat. No. MB-12) to minimize the background signal.
3. Sample Dilution: Dilute your antibody of interest from 20µg/mL to 0.039µg/mL in Assay buffer.
4. Add 100 µL beads to each tube or plate well. Place the beads on the magnetic separator for 1-2 min. Remove the supernatant.
5. Add 100 µL diluted sample to the pelleted beads, and mix the beads with samples by mixer. Add 100 µL of assay buffer as a blank control into wells containing the beads.
6. Cover the tubes on a rotator or place the plate on a plate mixer and incubate for 60 minutes at room temperature. Alternatively, rotate overnight at 4°C.
7. Place the tube/plate on the magnetic separator for 2 min. Remove the supernatant.
8. Remove the tube/plate from the magnetic separator and resuspend the pelleted beads in 200 µL of Assay/Washing Buffer by a vortex.
9. Wash the beads for a total of 4 times by repeating steps 7–8. After the last wash, remove the supernatant.
10. Dilute your Secondary Antibody in Assay buffer. Add 100µL secondary antibody (at an appropriate dilution ratio) to the beads. We recommend PE anti-Human IgG Fc (Biolegend, Cat. No. 409304) at 1:200 to detect your human IgG antibody samples. Any other fluorescent-labeled secondary antibody is appropriate.

11. Place the tubes on a rotator or place the plate on a plate mixer, and incubate for 60 minutes at room temperature. Cover with foil to avoid photobleaching.

12. Repeat steps 7-8 for a total of four washes with Assay/Washing buffer. After the last wash remove the supernatant.

13. Add 100 µL of Assay/Washing Buffer to the beads, and resuspend the Beads.

14. Transfer the 100µL beads into a 96-well black plate that is compatible with your plate reader. Keep the beads well suspended.

15. Read the plate at excitation 488 nm/emission 575 nm on a plate reader within 10 min (Avoid the precipitation of the beads).

■ Important Note

This product is for research use only and not intended for therapeutic or *in vivo* diagnostic use.

■ Contact Information

If you have any questions, please contact our technical support team at: TechSupport@acrobiosystems.com