

## Anti-EGFR aptamer, Magnetic Cell Isolation Kit

(Catalog No. EGFR-2369BCI/EGFR-2369FBCI)

### Description

AptSci EGFR cell isolation Kit (Catalog No. EGFR-2369FBCI) contains sufficient reagents for 10 reactions (up to  $0.4 \times 10^9$  total cells) and provides a bi-labeled aptamer that has FITC at 5'-end to monitor protein expression by flow cytometry and biotin at 3'-end to separate target cells. EGFR cell isolation kit (Catalog No. EGFR-2369BCI) contains biotinylated EGFR aptamer.

### Kit Contents

Reagent	Details
<sup>1</sup> Biotinylated EGFR aptamer (5'-Biotin), Biotinylated anti-human EGFR aptamer (MW: ~18 kDa) is supplied as a dried form	Quantity. 1 vial (~7.25 ug)
<sup>2</sup> Bi-labeled EGFR aptamer (5'-FITC, 3'-Biotin), FITC dye conjugated biotin anti-human EGFR aptamer (MW: ~18 kDa) is supplied as a dried form	Quantity. 1 vial (~7.25 ug)
Streptavidin Magnetic Beads, Beads are supplied in PBS pH 7.4 containing 0.01 % Tween-20, 0.09% NaN <sub>3</sub>	Quantity. 1 vial (0.1mL)
5X BB, binding buffer (filtered), pH 7.4	Quantity. 1 vial (8 mL)
1X RB, Releasing Buffer	Quantity. 1 vial (50uL)

Note: 1) Catalog No. EGFR-2369BCI, 2) Catalog No. EGFR-2369FBCI

### Reagent and instrument requirements

Magnetic stand  
 Rotating or Rocking mixer  
 Benchtop centrifuge  
 Phosphate buffered saline (PBS) pH 7.4 (Ca<sup>++</sup> and Mg<sup>++</sup>-free)  
 FBS or BSA(Bovine serum albumin)

### Storage/Stability

Store the kit at 2~8°C upon receipt and when not in use. Kit product is stable at 2~8°C for at least 1 year.

### Procedure

Note: This procedure describes the processing of  $1 \times 10^7$  total cells in 1.5 mL tubes. In the case of EGFR cell isolation Kit (Catalog No. EGFR-2369FBCI), all subsequent steps should be carried out in dark to prevent the fluorophore fading.

### Preparation of Materials

#### 1. Reconstitution of aptamer

Dissolve the stock aptamers completely in 100 µl of H<sub>2</sub>O. The reconstituted aptamer should be stored at -20°C to 4°C until use.

Note: The concentration of aptamer is 4 pmol/ µl

#### 2. Preparation of 1X BB containing 5% FBS

Dilute 5X BB and FBS to a final concentration with ultrapure H<sub>2</sub>O

Note: 5% FBS can be replaced by 0.5% BSA.

### Cell preparation

1. Prepare a single-cell suspension by standard methods depending on whether the cells are from tissues, blood, or cell cultures.
2. Count the cells using a hemacytometer.

### Cell isolation

Note: Cells and reagents should be kept at 2~8°C or on ice.

1. Suspend cell pellet in cold 1X BB containing 5% FBS at a density of  $1 \times 10^7$  cells/mL prior to starting the procedure.
2. Perform heating & cooling step for the regeneration of aptamer (see handling protocol). Briefly, 10 µl of aptamers are diluted in 10 µl of 2X BB and heated for 5 minutes at 95°C. The aptamer solution should be allowed to cool slowly to room temperature for 15 minutes.
3. Add aptamer and gently mix the cell, then incubate for 15 minutes at 2~8°C on a rocking mixer. At the end of the incubation period, centrifuge at 300 x g for 1~2 minutes and remove the supernatant.
4. Wash the cell suspension by adding 0.5 mL of cold PBS and centrifuge at 300 x g for 1~2 minutes. Remove the supernatant completely and gently resuspend the cell pellet with pipette in 1 mL of cold 1X BB containing 5% FBS.
5. During step 3, transfer 10 µl of streptavidin magnetic bead to fresh tube. Wash the beads by gently mixing in 1 ml of 1X BB containing 5% FBS for several minutes, and then place the tube on a magnet until the beads collect to the side of the tube wall and remove the supernatants.
6. Add the cell suspension to pre-washed streptavidin magnetic beads. Mix the cell pellet with pipette and incubate for 15 minutes at 2~8°C on a rocking mixer.
7. At the end of the incubation period, place the tube on a magnet, and then allow the bead complex (*magnetically tagged cells, positive cells*) to collect at the tube wall and transfer the supernatants (*Untagged cells, negative cells*) to new tube (*Negative fraction*).
8. Wash the bead complex (*magnetically tagged cells*) by gently pipetting up and down in 0.5 mL of cold PBS. Then place the tube on a magnet, and then allow the beads complex to collect at the tube wall and remove the supernatants.
9. Repeat the washing steps (step 8) 1~2 times.

Note: If purity of the cell selection is critical, increase washing volume up to 1mL

10. Remove the tube containing the magnetically selected cells from the magnet and resuspend cells briefly in 0.1 mL of PBS. This final magnetically isolated fraction contains the desired isolated EGFR<sup>+</sup> cells (*Positive fraction*). In order to release target cells from bead-bound cells, process to next section.

Note: For EGFR cell isolation kit (Catalog No. EGFR-2369FBCI), the magnetically selected EGFR<sup>+</sup> cells can be directly analyzed by flow cytometry. Resuspend the appropriate amount of selected cells in 100~500 µl of PBS and immediately apply to flow cytometry analysis without additional fluorescent dye-conjugated EGFR antibody or aptamer.

For EGFR cell isolation kit (Catalog No. EGFR-2369BCI), the magnetically selected EGFR<sup>+</sup> cells can be also analyzed with additional method. For flow cytometry, the appropriate amount of selected cells can be stained using dye-conjugated EGFR aptamer (refer to related products) or antibody.





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### Release of target cells

Aptsci Magnetic Cell Isolation kit provides a releasing method to obtain a bead-free and aptamer-free target cell.

1. Add 4  $\mu$ L of 1X RB to resuspended cell with 0.1 mL of pre-warmed PBS (37°C) and incubate for 15~20 minutes at room temperature with gentle mixing. For a higher elution efficiency, incubation time can be elongated.

*Note* : Excessive incubation time can affect the viability of cells.

2. Place the tube on a magnetic stand and transfer supernatant containing released target cells into new tube (Collection tube).

3. Resuspend the bead fraction in 100~200  $\mu$ L of pre-warmed PBS and repeat 2~3 times to maximize the cell releasing.

4. Centrifuge the tube containing released target cells at 300 x g for 1~2 minutes and remove the supernatant. Then resuspend the cells by adding PBS or cell culture media. This final fraction contains the desired a bead-free and aptamer-free EGFR<sup>+</sup> cells. The cells are now ready to be stained and used in other downstream applications.

### Related Products

Product	Catalog No.
Anti-EGFR aptamer (clone 2369), FITC Conjugate	2369FC-FITC
Anti-EGFR aptamer (clone 2369), Cy3 Conjugate	2369FC-Cy3
Anti-EGFR aptamer (clone 2369), Cy5 Conjugate	2369FC-Cy5
Anti-EGFR aptamer (clone 2369), Dy647 Conjugate	2369FC-Dy647
Anti-EGFR aptamer (clone 2369), TAMRA Conjugate	2369FC-TAMRA

#### UK & Rest of World

AMS Biotechnology (Europe) Ltd  
 184 Milton Park, Abingdon,  
 OX14 4SE, UK

#### Switzerland

AMS Biotechnology (Europe) Ltd  
 Centro Nord-Sud 2E  
 CH-6934, Bioggio-Lugano

#### North America

amsbio LLC -  
 1035 Cambridge Street  
 Cambridge, MA 02141

**www.amsbio.com | info@amsbio.com**



T: +44 (0) 1235 828200  
 F: +44 (0) 1235 820482



T: +49 (0) 69 779099  
 F: +49 (0) 69 13376880



T: +41 (0) 91 604 55 22  
 F: +41 (0) 91 605 17 85



T: +1 617 945 5033  
 F: +1 617 9458218



## Anti-EGFR aptamer, Magnetic Cell Isolation Kit (Catalog No. EGFR-2369BCI/ EGFR-2369FBCI)

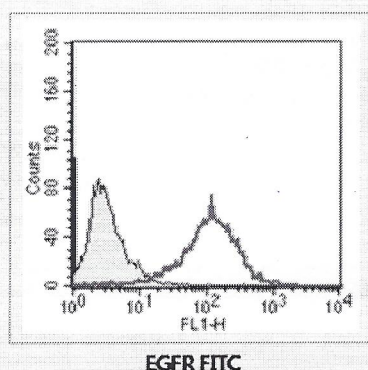
### Description

AptSci provides two types of kits. One is biotinylated aptamer based cell isolation kit (Catalog No. EGFR-2369BCI). The other is bi-labeled (FITC dye conjugated biotin aptamer) aptamer based cell isolation kit (Catalog No. EGFR-2369FBCI). Aptamer based magnetic cell isolation kit products do not adversely affect cells during isolation process, thus can be used to isolate pure, viable and functional cells which advance your biology research.

AptSci EGFR cell isolation kit is ideal for positive isolation of EGFR expressing target cells directly from all types of samples. Cell can also be eluted from bead-cell complexes with releasing buffer included in Kit, and then be used in all downstream experiments, including flow cytometry, cell culture and molecular studies.

### Component description

- AptSci aptamer is a single stranded oligonucleotide that is engineered through advanced SELEX with modified nucleotide.
- Aptamer is generated with recombinant human EGFR protein produced in mammalian cells and binds their cellular target with high affinity and specificity (Fig. 1).
- Magnetic beads are uniform, colloiddally stable and non-porous beads (1µm diameter) covalently coupled with streptavidin.
- Bi-labeled aptamer that has FITC at 5'-end to monitor protein expression by flow cytometry and biotin at 3'-end to separate target cells.



**Fig. 1. Flow cytometry histograms showing the binding of representative EGFR aptamer in A431 cells.**

Approximately  $1 \times 10^6$  cells were incubated with biotinylated EGFR aptamer and stained 2<sup>nd</sup> FITC-streptavidin (Pink histogram). As a control, the cells were stained 2<sup>nd</sup> FITC-streptavidin (Gray histogram).

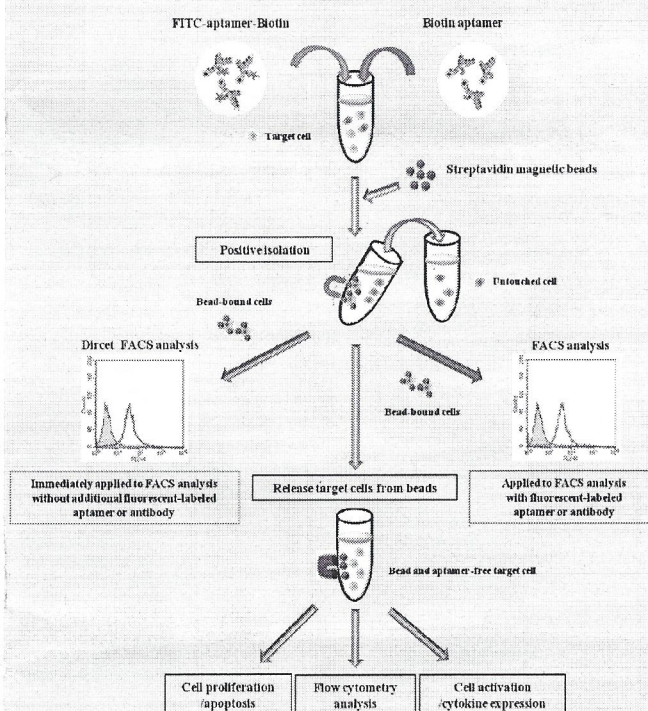
### Principle of the AptoPrep™ Cell Isolation

AptSci cell isolation kit is designed to isolate cells via an indirect method and for positive selection principle using biotinylated aptamers and streptavidin magnetic beads.

Target cells are specifically labeled with biotinylated aptamer against cell surface target of desired cells. Streptavidin magnetic beads allow for efficient binding to the aptamer labeled cell. Magnetically labeled target cells are then separated from unlabeled cells using magnet. FACS analysis can immediately be performed with bi-labeled aptamer during cell isolation process (Catalog No. EGFR-2369FBCI). In final step, bead-free and aptamer-free target cells were released from bead-bound cells (positive fraction) using releasing component (Fig. 2).

**Positive isolation:** Discard the supernatants and use the bead-bound cells for downstream application.

**Release target cells from beads:** Bead-bound cells are washed and target cells are released from the beads with releasing buffer included in Kit.



**Fig. 2. Overview of AptoSci cell isolation procedure.**

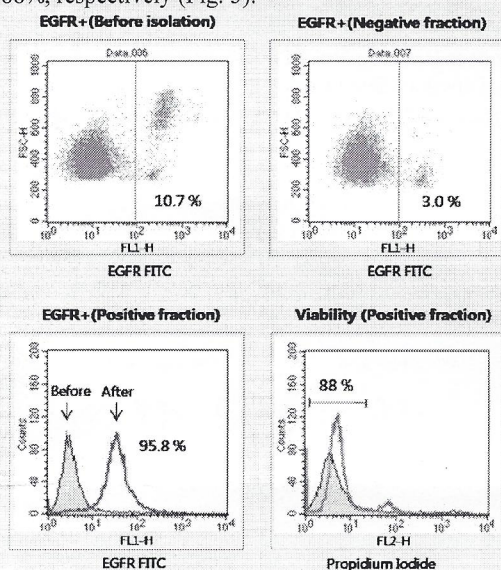




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### Typical results of the AptoPrep™ EGFR Cell Isolation

Isolation of EGFR<sup>+</sup> cells from EGFR<sup>-</sup> cells was performed with AptSci EGFR cell isolation kit. Human epidermoid carcinoma A431 cells (EGFR positive cell,  $\sim 1 \times 10^6$  cells) were spiked with lymphocytes (EGFR negative cell,  $1 \times 10^7$  cells). Yield of EGFR<sup>+</sup> cells isolation was measured at 71.9 %. Purity and viability of recovered EGFR<sup>+</sup> cells were measured at 95.8% and 88%, respectively (Fig. 3).

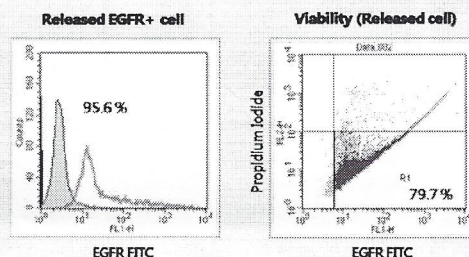


**Fig. 3. Isolation of EGFR<sup>+</sup> cells from EGFR<sup>-</sup> with AptSci EGFR cell isolation kit.** Both start sample before isolation and negative fraction after isolation were stained with biotinylated EGFR aptamer and 2<sup>nd</sup> FITC-streptavidin (Green dot plot). Positive fraction is stained with biotinylated EGFR aptamer and 2<sup>nd</sup> FITC-streptavidin (Pink histogram) and propidium iodide for cell viability (Blue histogram). Cell isolation was performed with biotinylated aptamer (Catalog No. EGFR-2369BCI). As a control, the cells were stained with FITC conjugated control aptamer.

### Downstream application

EGFR<sup>+</sup> cells can be efficiently isolated from a sample with AptSci EGFR cell isolation kit. Lyse the cells directly after isolation, and isolate proteins, DNA, or mRNA to be used in PCR, microarrays, proteomics, and other applications where the removal of beads is not required. For functional studies such as cytokine expression, proliferation/apoptosis induction or for flow cytometry analysis, the cells need to be released from beads after positive isolation of cell. Releasing buffer included in Kit will allow you to collect the bead-free and aptamer-free EGFR<sup>+</sup> cell.

After elution of EGFR<sup>+</sup> cells with releasing buffer, elution yield was calculated by counting cells with hemocytometer and measured at 51% (data not shown). Purity and viability of released EGFR<sup>+</sup> cells were measured at 95.6% and 79.7%, respectively (Fig. 4).



**Fig. 4. Flow cytometry showing bead-free and aptamer-free EGFR<sup>+</sup> cells** Released cell fraction (Green histogram and Green dot plot) was stained with biotinylated EGFR aptamer and 2<sup>nd</sup> FITC-streptavidin and propidium iodide for cell viability (Red dot plot). Elution of target cell was performed with biotinylated aptamer (Catalog No. EGFR-2369BCI). As a control, the cells were stained with FITC conjugated control aptamer (Gray histogram).

### Product Information

- **Product name:** Anti-EGFR aptamer, Magnetic Cell Isolation Kit
- **Catalog number:** EGFR-2369BCI (biotinylated aptamer based cell isolation kit), EGFR-2369FBCI (bi-labeled aptamer based cell isolation kit)
- **Content:** EGFR-2369BCI (Biotinylated aptamer, streptavidin-coated magnetic bead and buffer), EGFR-2369FBCI (FITC dye conjugated biotin aptamer, streptavidin-coated magnetic bead and buffer)
- **Form:** Dried aptamer and bead in PBS pH 7.4 containing 0.01 % Tween-20, 0.09% NaN<sub>3</sub>
- **Protein source for generation of aptamer:** Recombinant protein produced in mammalian cells.
- **Specificity:** Anti-EGFR aptamer binds to human EGFR. Cross reactivity with other species has not been tested.
- **MW:** ~18 kDa
- **Tested applications:** FACS and cell isolation
- **Shipping & Storage :** At 2°C to 8°C. There is no decrease in performance of the kit after storage for 1 year at 2°C to 8°C.

**UK & Rest of World**  
 AMS Biotechnology (Europe) Ltd  
 184 Milton Park, Abingdon,  
 OX14 4SE, UK

**Switzerland**  
 AMS Biotechnology (Europe) Ltd  
 Centro Nord-Sud 2E  
 CH-6934, Bioggio-Lugano

**North America**  
 amsbio LLC -  
 1035 Cambridge Street  
 Cambridge, MA 02141

**www.amsbio.com | info@amsbio.com**



T: +44 (0) 1235 828200  
 F: +44 (0) 1235 820482



T: +49 (0) 69 779099  
 F: +49 (0) 69 13376880



T: +41 (0) 91 604 55 22  
 F: +41 (0) 91 605 17 85



T: +1 617 945 5033  
 F: +1 617 9458218