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 x 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
[1]**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)
[2]**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% NaN3	**Quantity.** 1 vial (0.1mL)
5X BB, binding buffer (filtered), pH 7.4	**Quantity.** 1 vial (8mL)
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^{++} and Mg^{++}-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 x 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_2O. 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_2O
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 x 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 $EGFR^+$ 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 $EGFR^+$ 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 $EGFR^+$ 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 µl 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 µl 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 $EGFR^+$ cells. The cells are now ready to be stained and used in other downstream applications.

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Typical results of the AptoPrep™ EGFR Cell Isolation

Isolation of EGFR+ cells from lymphocytes was performed with AptSci EGFR cell isolation kit. Human epidermoid carcinoma A431 cells (EGFR positive cell, ~1x10^6 cells) were spiked with lymphocytes (EGFR negative cell, 1x10^7 cells). Yield of EGFR+ cells isolation was measured at 71.9 %. Purity and viability of recovered EGFR+ cells were measured at 95.8% and 88%, respectively (Fig. 3).

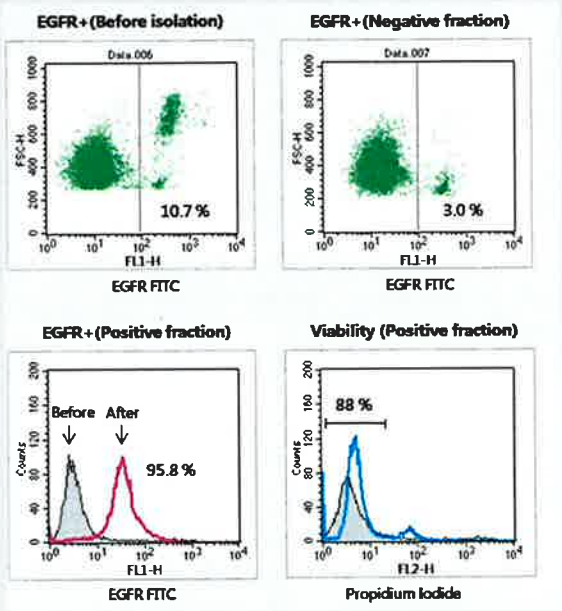

Fig. 3. Isolation of EGFR+ cells from lymphocytes with AptSci EGFR cell isolation kit.
Both start sample before isolation and negative fraction after isolation were stained with FITC-human EGFR aptamer (Green dot plot). Positive fraction is stained with FITC-human EGFR aptamer (Pink histogram) and propidium iodide for cell viability (Blue histogram). As a control, the cells were stained with FITC conjugated control aptamer.

Downstream application

EGFR+ 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+ cell.
After elution of EGFR+ 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+ cells were measured at 95.6% and 79.7%, respectively (Fig. 4).

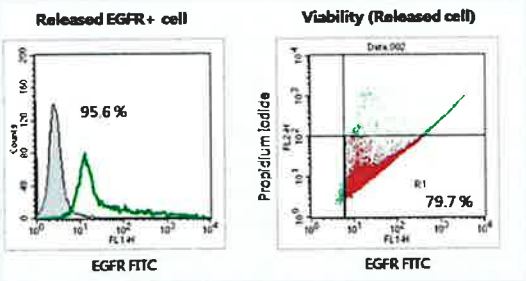

Fig. 4. Flow cytometry showing bead-free and aptamer-free EGFR+ cells are released with releasing buffer.
Released cell fraction (Green histogram and Green dot plot) was stained with human FITC-EGFR aptamer and propidium iodide for cell viability(Red dot plot). As a control, the cells were stained with 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 50 mM Tris pH8.0, 150 mM NaCl, 0.05% NaN_3.
- **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.

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, colloidally 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.

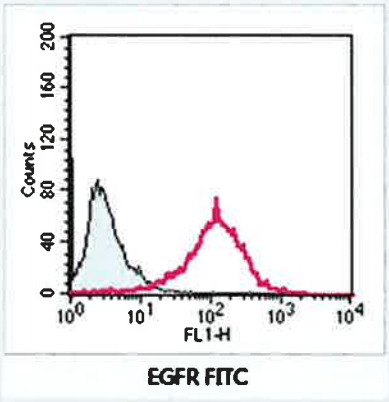

Fig. 1. Flow cytometry histograms showing the binding of representative EGFR aptamer in A431 cells.
Approximately 1 × 10^6 cells were incubated with biotinylated EGFR aptamer and stained 2nd FITC-streptavidin (Pink histogram). As a control, the cells were stained 2nd FITC-streptavidin (Gray histogram).

Principle of the AptoPrep™ Cell Isolation

AptSci cell isolation kit is designed to isolate cells via a 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.

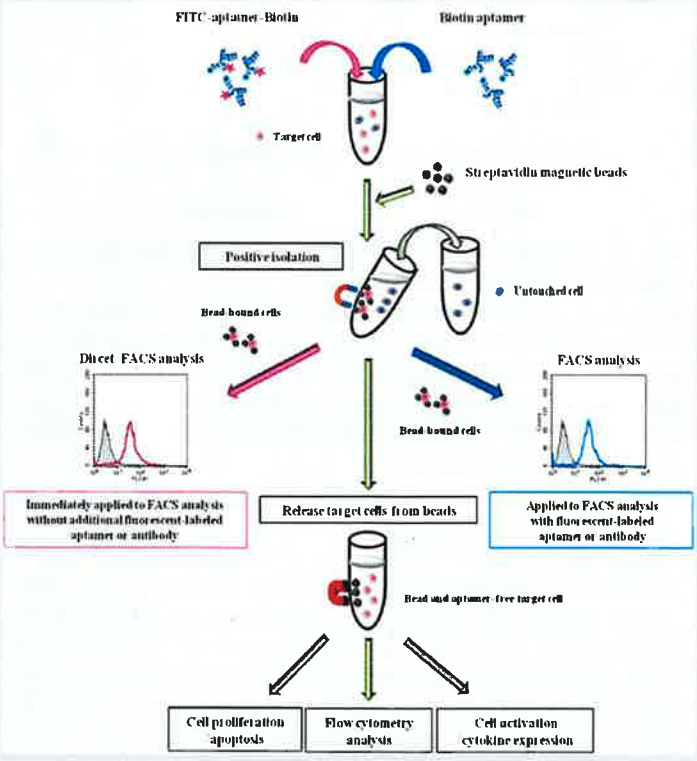

Fig. 2. Overview of AptSci cell isolation procedure

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General Handling Guide for Aptamers

Instructions

* If not ordered otherwise, all custom Aptsci aptamers are ready for use upon resuspension with the exception of thiol-modified aptamers (see **REDUCTION PROTOCOL** on next page). Aptamers are shipped in room temperature as a dried form and are attached to the container wall like a film. Appearance of aptamers may transparent.
* Aptamers are stable at neutral pH range (7.0 - 8.0).
* Aptamers labeled with light-sensitive dyes (such as Cy3) or PC-linker are provided in opaque brown container to protect their activity.

Resuspension

1. Before open the container, always briefly **SPIN DOWN** for the first time after delivery to avoid loss of the aptamer pellet.
2. Dissolve the stock aptamers completely to the desired stock concentration with buffers or purified water, by shaking.
   - DNase free purified water or any biological buffers (such as PBS, HEPES, Tris, etc.) are suitable. For the dilution of aptamers, please use proper buffer upon your experimental condition. The recommended diluent volume is 100ul~1ml. The concentration depending on your application and the yield of the resulting product.
   - Addition of divalent cation such as magnesium in buffer is optional for maintaining proper structure of aptamer. **(Final 1 ~ 5mM $MgCl_2$ is recommended)**
3. Aliquot and store stock aptamers at -20°C to -70°C until you use it.
   - Make a stock solution and working aliquots which should be thawed relatively infrequently.

Heating & cooling (H&C) step

1. Before every use, perform Heating & cooling (H&C) step for the PROPER FOLDING of aptamer structure in buffer including 1~5mM $MgCl_2$
2. Please heat aptamers in proper buffer solution at **95°C for 5 min.**, and then leave the tubes on the **bench for 15 min.**

Storage

* Aptamers are stable in solution of neutral pH at 4°C. Properly reconstituted aptamers stored at -20°C to -70°C should be stable.
* For long term use, aliquoting is recommended. Please keep the aliquots at -20°C to -70°C and avoid freeze-thaw cycles.
* Aptamers labeled with light-sensitive dyes or PC-linker could lose their ability over time, please keep in dark place.

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Reduction protocol for thiol aptamer

If you want to use thiol aptamer, we recommend to have a reduction step prior to each use.

1. Dissolve thiol aptamer in reducing solution
   e.g. 292.5ul of DW, 7.5ul of 2M Triethylammonium acetate, 3ul of 1M DTT, 3ul of Triethylamine
2. Incubate at Room temperature for 1.5 hours
3. Perform Desalting step and buffer exchange with Centrifugal Filter, Ethanol precipitation or HPLC

FAQ

1. What is the size of Aptsci aptamers?
- Please refer to the product report delivered with Aptsci aptamers. In general, Aptsci aptamer consists of less than 76 bases and is ~25kDa in size.

2. Can aptamer be used in the same applications as antibodies?
- Aptamer can be used for the typical antibody mediated methods such as immunofluorescence, flow cytometry and immunoprecipitation. Aptamer can also be used for double staining together with an antibody provided that aptamer and antibody is not directed against the same epitope on the antigen.

3. What is the recommended range of pH?
- Aptamer is stable at neutral pH range (pH7.0 - 8.0). Aptamer may degrade in low pH buffer solution.

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