Anti-ERBB2 aptamer, Magnetic Cell Isolation Kit
(Catalog No. ERBB2-1194BCI/ERBB2-1194FBCI)

Description

AptSci ERBB2 cell isolation Kit (Catalog No. ERBB2-1194FBCI) contains sufficient reagents for 40 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. ERBB2 cell isolation kit (Catalog No. ERBB2-1194BCI) contains biotinylated ERBB2 aptamer.

Kit Contents

Reagent	Details
[1)]**Biotinylated ERBB2 aptamer (5′-Biotin),** Biotinylated anti-human ERBB2 aptamer (MW: ~15 kDa) is supplied as a dried form	**Quantity.** 1 vial (~24 ug)
[2)]**Bi-labeled ERBB2 aptamer (5′-FITC, 3'-Biotin),** FITC dye conjugated biotin anti-human ERBB2 aptamer (MW: ~15 kDa) is supplied as a dried form	**Quantity.** 1 vial (~24 ug)
Streptavidin Magnetic Beads, Beads are supplied in 50 mM Tris pH8.0, 150 mM NaCl, 0.05% NaN_3	**Quantity.** 1 vial (0.4mL)
5X BB, binding buffer (filtered), pH 7.4	**Quantity.** 1 vial (32 mL)
50X RB, Releasing Buffer	**Quantity.** 1 vial (0.2 mL)

Note: 1) Catalog No. ERBB2-1194BCI, 2) Catalog No. ERBB2-1194FBCI

Reagent and instrument requirements

Magnetic stand
Rotating or Rocking mixer
Benchtop centrifuge
Phosphate buffered saline (PBS) pH 7.4 (Ca^{++} or 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 ERBB2 cell isolation Kit (Catalog No. ERBB2-1194FBCI), 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 400 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. Suspend the streptavidin magnetic beads with 15 µl of 1X BB.
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 $ERBB2^+$ cells (*Positive fraction*). In order to release target cells from bead-bound cells, process to next section.
Note: For ERBB2 cell isolation kit (Catalog No. ERBB2-1194FBCI) , the magnetically selected $ERBB2^+$ 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 ERBB2 antibody or aptamer.
For ERBB2 cell isolation kit (Catalog No. ERBB2-1194BCI), the magnetically selected $ERBB2^+$ cells can be also analyzed with additional method. For flow cytometry, the appropriate amount of selected cells can be stained using dye-conjugated ERBB2 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 ERBB2^{+} cells. The cells are now ready to be stained and used in other downstream applications.

Related Products

Product	Catalog No.
Anti-ERBB2 aptamer (clone 1194), FITC Conjugate	1194FC-FITC
Anti-ERBB2 aptamer (clone 1194), Cy3 Conjugate	1194FC-Cy3
Anti-ERBB2 aptamer (clone 1194), Cy5 Conjugate	1194FC-Cy5
Anti-ERBB2 aptamer (clone 1194), Dy647 Conjugate	1194FC-Dy647
Anti-ERBB2 aptamer (clone 1194), TAMRA Conjugate	1194FC-TAMRA

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