Anti-PECAM (CD31) DNA aptamer, Magnetic Cell Isolation Kit
(Catalog No. CD31-2196BCI/CD31-2196FBCI)

Description

AMSBIO CD31 cell isolation Kit (Catalog No. CD31-2196FBCI) containssufficient reagents for 40 reactions (up to 0.4 x 10^9 total cells) andprovides a bis-labeled aptamer that has Cy5 at 5'-end to monitor proteinexpression by flow cytometry and biotin at 3'-end to separate targetcells. CD31 cell isolation kit (Catalog No. CD31-2196BCI) contains
biotinylated CD31 aptamer.

Kit Contents

Reagent	Details
[1]**Biotinylated CD31 aptamer (5'-Biotin),** Biotinylated anti-human CD31 aptamer (MW: ~25 kDa) is supplied as a dried form	**Quantity.** 1 vial (~41 ug)
[2]**Bis-labeled CD31 aptamer (5'-FITC, 3'-Biotin),** FITC dye conjugated biotin anti-human CD31 aptamer (MW: ~25 kDa) is supplied as a dried form	**Quantity.** 1 vial (~41 ug)
Streptavidin Magnetic Beads, Beads are supplied in PBS pH 7.4 containing 0.01 % Tween-20, 0.09% NaN3	**Quantity.** 1 vial (0.4mL)
5X BB, binding buffer (filtered), pH 7.4	**Quantity.** 1 vial (32 mL)
1X RB, Releasing Buffer	**Quantity.** 1 vial (0.2 mL)

Note: 1) Catalog No. CD31-2196BCI, 2) Catalog No. CD31-2196FBCI

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 CD31 cell isolation Kit (Catalog No. CD31-2196FBCI), 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 hemocytometer.

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 and 20-200 µM dextran sulfate 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 $CD31^+$ cells (*Positive fraction*). In order to release target cells from bead-bound cells, process to next section.
Note: For CD31 cell isolation kit (Catalog No. CD31-2196FBCI) , the magnetically selected $CD31^+$ 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 CD31 antibody or aptamer.
For CD31 cell isolation kit (Catalog No. CD31-2196BCI), the magnetically selected $CD31^+$ cells can be also analyzed with additional method. For flow cytometry, the appropriate amount of selected cells can be stained using dye-conjugated CD31 aptamer (refer to related products) or antibody.

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Release of target cells

AMSBIO Magnetic Cell Isolation kit provides a releasing method toobtain 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 $CD31^+$ cells. The cells are now ready to be stained and used in other downstream applications.

Related Products

Product	Catalog No.
Anti-CD31 aptamer (clone 2196), FITC Conjugate	2196FC-FITC
Anti-CD31 aptamer (clone 2196), Cy3 Conjugate	2196FC-Cy3
Anti-CD31 aptamer (clone 2196), Cy5 Conjugate	2196FC-Cy5
Anti-CD31 aptamer (clone 2196), Dy647 Conjugate	2196FC-Dy647
Anti-CD31 aptamer (clone 2196), TAMRA Conjugate	2196FC-TAMRA

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