COSMO BIO CO., LTD.

Magnetic Cell separation Kit for Human CD44v9+ Cancer Stem Cell

Cat. No. CSC-SEP1

Edition Date: 2020/4/1

www.cosmobiousa.com

【 I 】 Introduction

- This product is a magnetic bead conjugated to rat-derived anti human CD44v9 monoclonal antibody (clone number : RV3)[1].
- Simply mix beads and cells to easily collect CD44v9-positive cells by magnetic separation.
- No need for large cell sorting equipment or complicated operations, just work on the bench top.
- CD44v9-positive population contains cancer stem cell rich, therefore CD44v9 beads are a useful tool for cancer stem cell research[2)-5)].
- For details, please see RV3 antibody description (Cosmo Bio Co., Ltd., #LKG-M001 or #LKG-M003).

【 II 】 Kit Components

Size: 10 tests

Storage temperature: 4 to 8° C. Do not freeze.

	Component	Volume	Quantity
1	anti-CD44v9 magnetic beads (RV3 beads)	200 µL	1 tube
2	Binding buffer	30 mL	1 bottle
3	Wash buffer	50 mL	1 bottle

The buffers and reagents in this kit are filter sterilized, and does not contain preservatives.

【III】 Other requirements (equipment and regent)

- Magnetic stand
- Centrifuge
- Vortex mixer
- Buffers (See next section)

【IV】Typical procedure (of cell separation)

【IV -1】Beads preparation

* experiments take place in ambient condition unless temperature indicated
* bring Binding and Wash Buffer to room temperature before use.

1. Preliminarily well disperse the RV3 beads by vortex mixer or pipetting, taking care to avoid foam formation.
2. Dispend 20 μL of RV3 beads into 200 μL of Binding buffer.
3. Vortex gently and spin down, then put on magnetic stand (up to about 5 minutes).
4. Remove supernatant and add 200 μL of Binding buffer.
5. Repeat step 3 and 4 once.
(Wash with Binding buffer total in twice.)
6. Resuspend RV3 beads in 50 μL of Binding buffer after the second wash with Binding buffer.

【IV -2】Cells preparation

1. Wash cells with Binding buffer and centrifuge at 300×g for 3 minutes. Aspirate supernatant.
2. Resuspend cell pellet in 50 μL of Binding buffer per up to $1x10^7$ total cells.

【IV -3】Binding and magnetic separation

1. Gently mix well RV3 beads and cell suspension.
2. Reaction for 20 minutes in 4°C refrigerator (tapping gently every 5 minutes).
3. Add 1mL of Wash buffer and centrifuge at 300×g, 3minutes. Aspirate supernatant.
4. Resuspend in 1mL of Wash buffer, vortex gently and spin down, then put on magnetic stand (up to about 5 minutes).
5. Aspirate supernatant completely.
6. Repeat step 4 and 5 twice.
(Wash with Wash buffer total in three times.)
7. Resuspend cells with a buffer suitable for the next step.

【V】Note

- All mixing steps should be done gently to avoid foaming.
- For optimal performance it is important to obtain a single-cell suspension. We recommend passing the cells through cell strainers (Falcon™ 40μm, #352340) to remove cell clumps.

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【VI】Experimental examples

【VI－ 1】Stable cell line expressing CD44v9-GFP

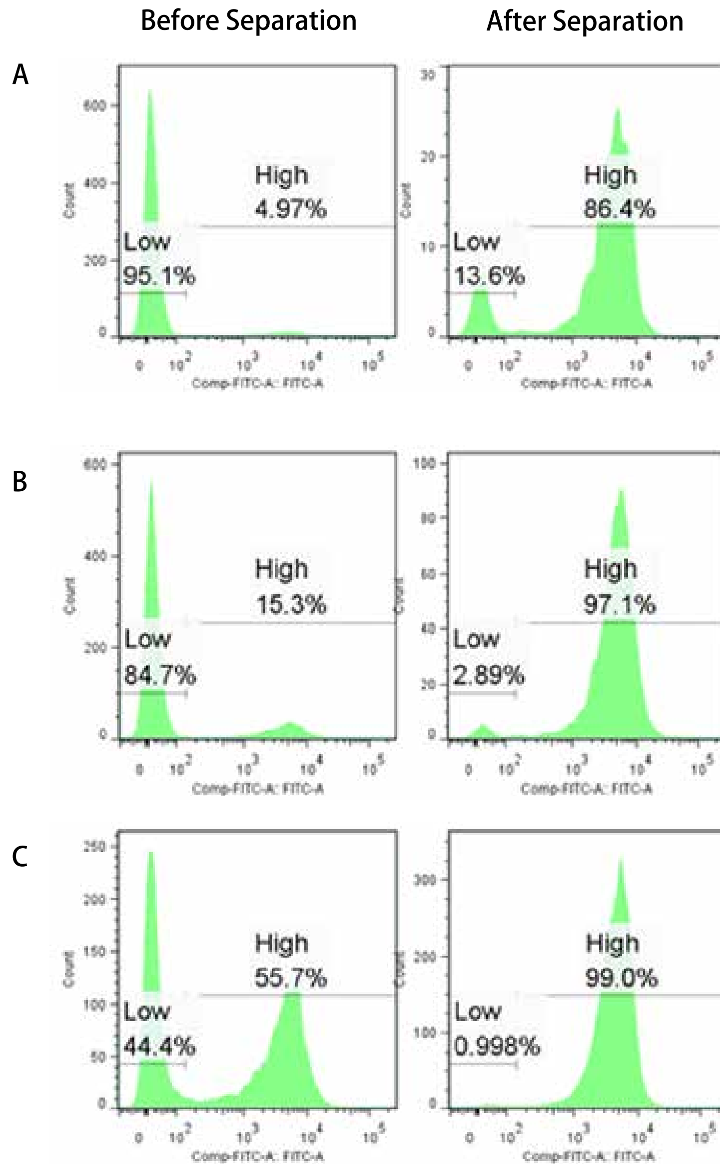

Fig.1 Percentage of CD44v9-expressing cells in RV3 bead-positive cells

A stable cell line expressing CD44v9-GFP and its parental 293F cell line were mixed at an arbitrary ratio, and then RV3 positive cells were separated. Cells were analyzed by flow cytometry. CD44v9-expressing cells were concentrated from A. 4.97%, B. 15.3%, and C. 55.7% to 86.4%, 97.1%, and 99.0%, respectively.

【VI -2】Human cancer cell lines

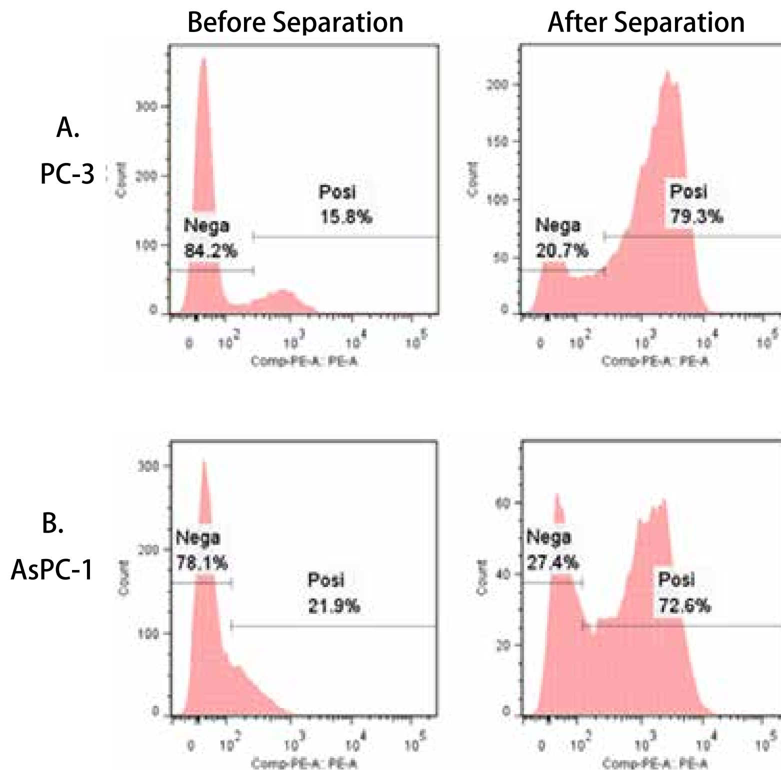

Fig.2 Percentage of CD44v9-expressing cells in human cancer cell lines

Separated RV3 bead-positive cells were stained with PE-labeled anti rat IgG antibody (secondary antibody) and analyzed by flow cytometry. In the two cancer cell lines, the proportion of CD44v9-expressing cells increased after CD44v9 bead separation.

A. PC-3: prostate cancer cell line. B. AsPC-1: pancreatic cancer cell line.

【VII】Example of Results

[1] Tanabe KK., et al., Lancet 1993; 341: 725–726. PMID: 8095628

[2] Nagano O., et al., Oncogene 21 January 2013, 1-8. PMID:23334333

[3] Ishimoto T., et al., Cancer Cell 2011; 19: 387–400. PMID : 21397861

[4] Tsugawa H., et al., Cell Host Microbe 2012 ; 12: 764-777. PMID: 23245321

[5] Yae T., et al., Nat Commun 2012; 3: 883: 1-9. PMID: 22673910

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