



Magnetic Cell separation Kit for Human CD44v9+ Cancer Stem Cell

Cat. No. CSC-SEP1

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【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 research²⁾⁻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 reagent)

- 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 Dispense 20 μ L of RV3 beads into 200 μ L 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 μ L of Binding buffer.

5 Repeat step 3 and 4 once.

(Wash with Binding buffer total in twice.)

6 Resuspend RV3 beads in 50 μ L of Binding buffer after the second wash with Binding buffer.

【IV -2】 Cells preparation

1 Wash cells with Binding buffer and centrifuge at 300 \times g for 3 minutes. Aspirate supernatant.

2 Resuspend cell pellet in 50 μ L of Binding buffer per up to 1×10^7 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 \times 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™ 40 μ m, #352340) to remove cell clumps.

【VI】 Experimental examples

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

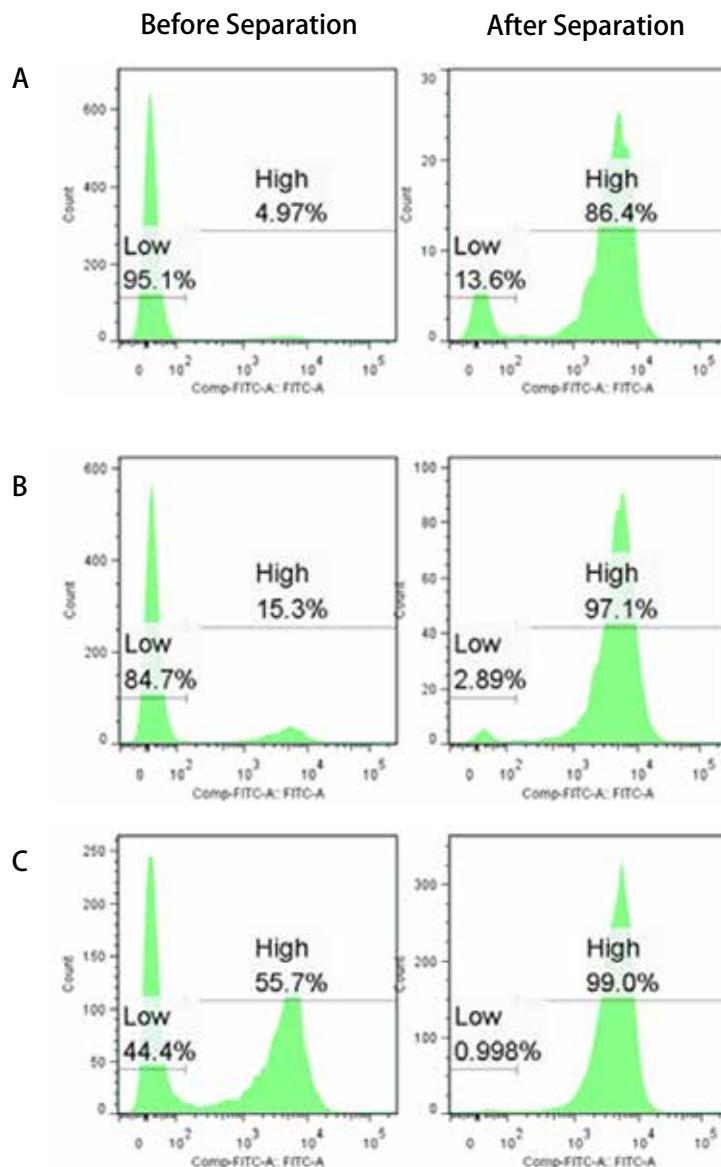


Fig.1 Percentage of CD44v9-expressing cells in RV3 bead-positive cells

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

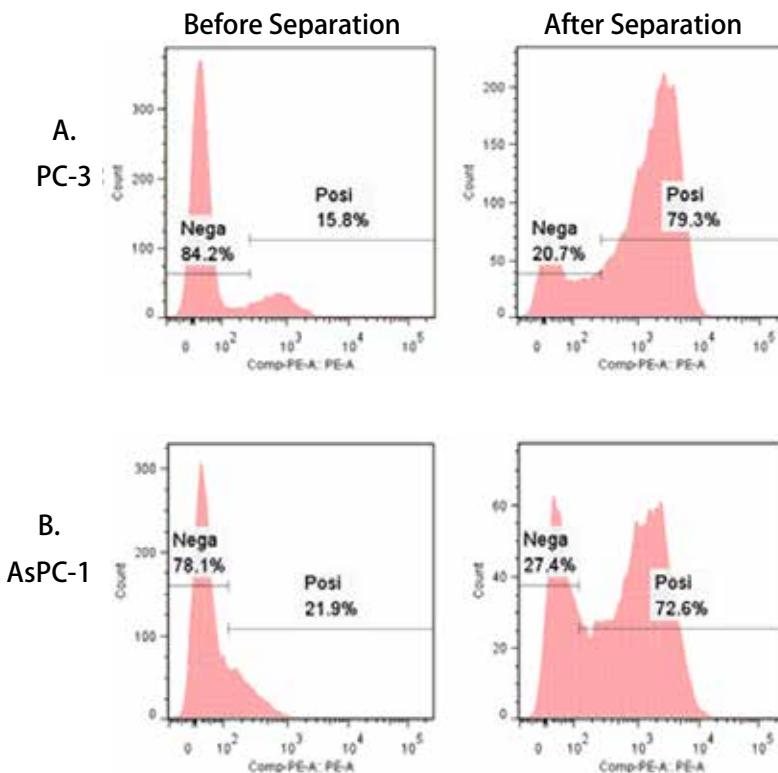


Fig.2 Percentage of CD44v9-expressing cells in human cancer cell lines

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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