

# 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)<sup>1)</sup>.
- 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<sup>2)-5)</sup>.
- 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  $\mu$ L of RV3 beads into 200  $\mu$ 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  $\mu$ L of Binding buffer.
- 5 Repeat step 3 and 4 once.  
(Wash with Binding buffer total in twice.)
- 6 Resuspend RV3 beads in 50  $\mu$ 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  $\mu$ L of Binding buffer per up to 1 $\times$ 10<sup>7</sup> 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 $\mu$ m, #352340) to remove cell clumps.

## 【VI】 Experimental examples

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

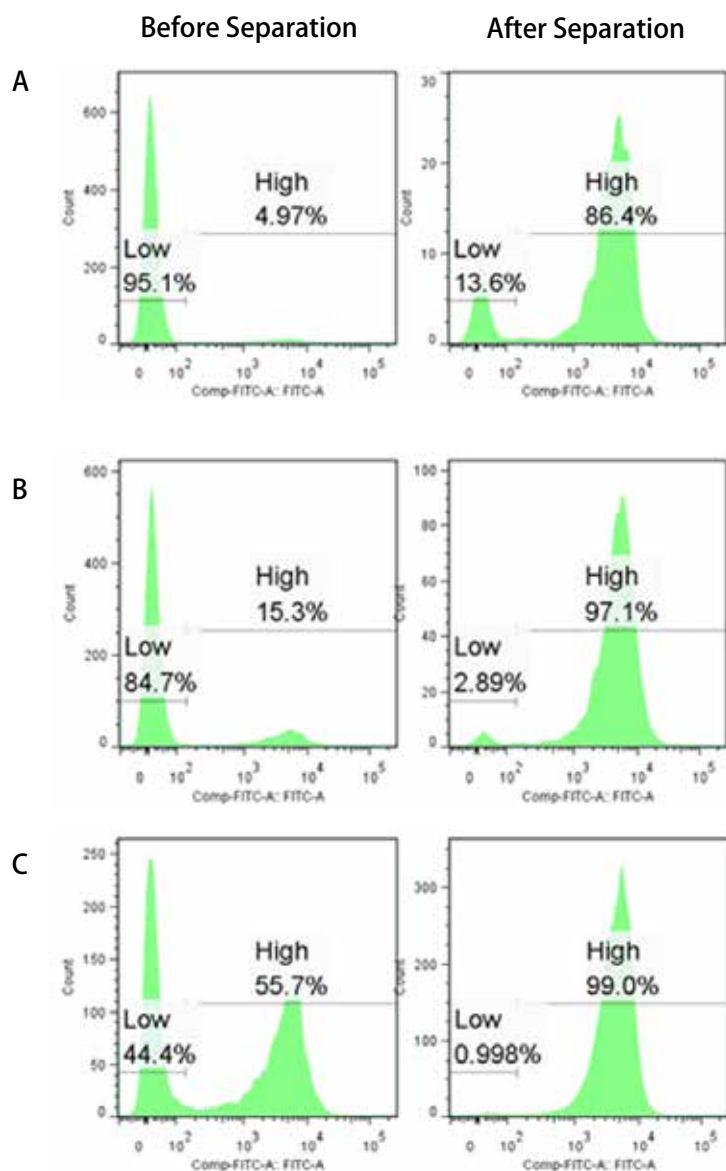


Fig.1 Percentage of CD44v9-expressing cells in RV3 bead-positive cells

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

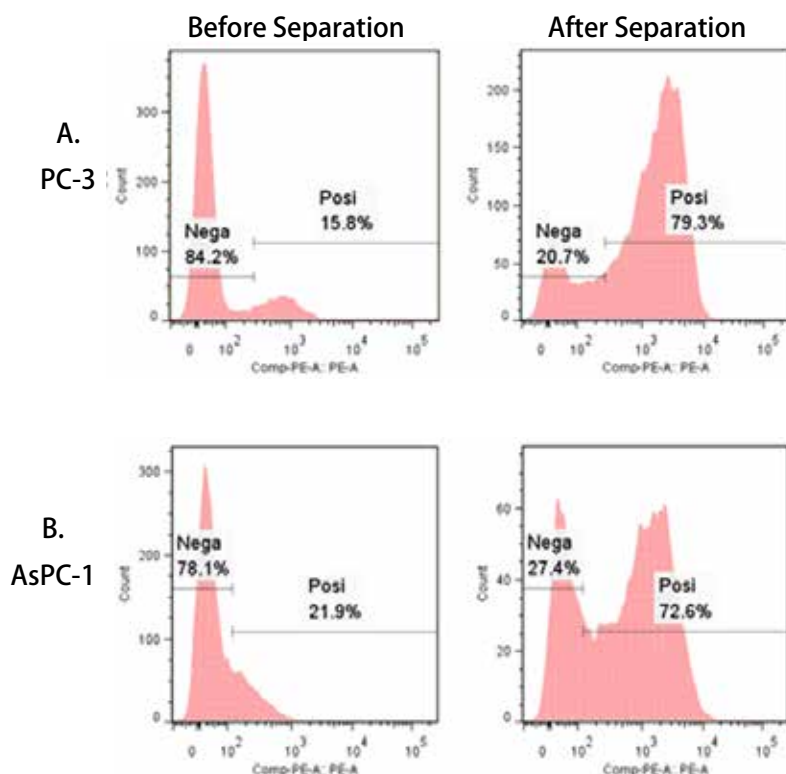


Fig.2 Percentage of CD44v9-expressing cells in human cancer cell lines

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



COSMO BIO CO., LTD.

【JAPAN】  
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,  
KOTO-KU. TOKYO 135-0016, JAPAN  
Phone: +81-3-5632-9610  
FAX: +81-3-5632-9619  
URL: <https://www.cosmobio.co.jp/>



COSMO BIO USA

【Outside Japan】  
2792 Loker Ave West, Suite 101  
Carlsbad, CA 92010, USA  
email: [support@cosmobiousa.com](mailto:support@cosmobiousa.com)  
URL: [www.cosmobiousa.com](http://www.cosmobiousa.com)  
Phone/FAX: (+1) 760-431-4600