ActSep® CD3/CD28 Separation & Activation Magnetic Beads

Size / Cat.No.: 1mL / GMP-TL603-1000
5mL / GMP-TL603-5000

Product Name

Generic Name	ActSep® CD3/CD28 Separation & Activation Magnetic Beads

Product Information

Consistence	2×10^8 beads / mL
Endotoxin	< 0.5 EU/mL
Reactive species	human
Storage Temperature	2-8℃
Validity period	24 months

Product Description

ActSep® CD3/CD28 Separation & Activation Magnetic Beads are suitable for separation of human $CD3^+$ T cells, providing a simple method for activating and expanding human T cells without the need for antigen presenting cells and antigens. By coupling anti-human CD3 and anti-human CD28 antibodies on magnetic beads, the primary and co-stimulatory signals needed to regulate T cell activation and expansion are provided. The beads are suitable for human T cell separation, activation and expansion, CAR-T and other T cell culture applications.

Protocol

1. PBMC cell preparation:

1.1 Resuspend human PBMC cells in PBS buffer containing 1% HSA and determine the percentage of CD3+ T cells in the sample by flow cytometry.

1.2 According to the CD3 positive rate of PBMC cells, adjust the density of $CD3^+$ T cells to 1×10^7 cells/mL with PBS buffer containing 1% HSA.

2. Wash ActSep® CD3/CD28 separation & activation magnetic beads:

2.1 Resuspend the magnetic beads (vortex for more than 30 seconds, or tilt and rotate for 5 minutes).

2.2 According to the number of magnetic beads and $CD3^+$ T cells, the recommended bead: T cell ratio is 1:1. Transfer the magnetic beads of a certain volume into the centrifugal tube. (In practical applications, customers can adjust the

ratio in the range of 1:1~ 3:1 according to the actual situation. With the increase of the number of magnetic beads, the separation efficiency of $CD3^+$T cells will be improved, and the activation and expansion of cells will be different).

2.3 Add 1mL of PBS buffer containing 1% HSA, then resuspend and vortex.

2.4 Place the tube on a magnet for 1 min, then discard the supernatant. Be careful not to inhale the magnetic beads.

3. Separation and activation:

3.1 Add PBMC adjusted $CD3^+$ T cell density to the above washed magnetic beads in proportion, and mix well.

3.2 Place the above centrifuge tube on a sample mixer with a rotating speed of 15~30 rpm and incubate at room temperature for 30 min.

3.3 Put the above centrifuge tube on the magnet for 1 min and discard the supernatant. (Or collect the supernatant. Count the cells and stain with anti-CD3 antibody for flow cytometric analysis to calculate separation efficiency in an indirect way.)

3.4 Resuspend the mixture of magnetic beads and cells with a complete medium containing 200 IU/mL rh IL-2 (Customers can make adjustments according to their actual experimental conditions, and determine whether to use autologous plasma), and adjust the cell density to $0.8\sim1.5\times10^6$/mL.

4. Expansion and culture:

Observe the cell status and supplement expansion medium regularly. When cell density is $>2.5\times10^6$ cells/mL or medium turns yellow, dilute the cells to approximately $0.5\sim1\times10^6$ T cells/mL. Cells can be harvested after culturing for an appropriate length of time (usually 12-14 days).

Notice:

1. After cell expansion, the cell suspension in the culture vessel should be gently blown periodically to allow adequate dispersion of the magnetic beads and cells.
2. During the culture process, the cells could be counted every 2 to 3 days, and the magnetic beads should be removed before cell counting. The magnetic beads removal process: the cells in the culture vessels were evenly suspended and sampled into the centrifuge tube, placed on the magnet, left for 1 min, and then transfer the supernatant to a new centrifuge tube.
3. After the cells were cultured for a suitable number of days, the cells needed to be harvested after removing the magnetic beads.
4. The purity, phenotype and activation state of T cells could be detected by flow cytometry during cell culture.(e.g. after 48 hours of cell culture, test the percentage of $CD69^+CD25^+$ on the cell membrane after removing the beads to evaluate the activation effect of magnetic beads.)

References

1. David M Barrett, Nathan Singh, Xiaojun Liu, Shuguang Jiang, Carl H June, Stephan A Grupp, Yangbing Zhao（2014）Relation of clinical culture method to T-cell memory status and efficacy in xenograft models of adoptive immunotherapy. Cytotherapy. May;16(5):619-30.

2. Barbara Tumaini, Daniel W Lee, Tasha Lin, Luciano Castiello, David F Stroncek, Crystal Mackall, Alan Wayne, Marianna Sabatino. (2013) Simplified process for the production of anti-CD19-CAR-engineered T cells. Cytotherapy 15:1406-1415.