

ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm)

Cat. No. MBS-C028

● Product Information

| Product | Size | Amount |
|--|--------------------|-------------------------|
| ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm) | 2.5 mg | 7.5×10^5 beads |
| | 10 mg (2.5 mg × 4) | 3.0×10^6 beads |

● Product Description

ActiveMax® VCAM-1 μBeads, premium grade (for cells) are uniform, superparamagnetic beads of 20 μm in diameter immobilized with Human VCAM-1 Protein, expressed from human 293 cells (HEK293) and contains AA Phe 25 - Glu 698 (Accession # P19320-1). ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm) are produced under sterile manufacturing conditions (ISO 5), and no animal- or human-derived components are used throughout the production process. It is produced under our rigorous quality control system that includes a comprehensive set of tests including sterility and endotoxin tests.

● Product Applications

ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm) is designed to synergistically activate Notch signaling in hematopoietic stem/progenitor cells (HSPCs). The synergistic interactions between Notch ligand Delta-like 4 and VCAM-1 are leveraged to enhance Notch signaling to support efficient T-cell lineage commitment, so the beads is recommended to use for in vitro production of induced pluripotent stem cell derived T cells coordinating with ActiveMax® Human DLL4 μBeads, premium grade (for cells, 20 μm) (Cat. No. MBS-C024). *The Product performance has been carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage. For use in clinical phases, we also offer a custom GMP protein service that tailors to your needs. We will work with you to customize and develop a GMP-grade product in accordance with your requests that also meets the requirements for raw and ancillary materials use in cell manufacturing of cell-based therapies.*

● Formulation

Lyophilized in PBS with 0.1% HSA, pH 7.4. Trehalose is added as protectant before lyophilization.

● Reconstitution

Please see Certificate of Analysis for specific instructions.
For best performance, we strongly recommend you to follow the reconstitution protocol provided in the Certificate of Analysis.

● Storage

This product is stable in storage under the following conditions:

- 20°C for 12 months in lyophilized state.
- 70°C for 3 months under sterile conditions after reconstitution.

Please avoid repeated freeze-thaw cycles after reconstitution. Immediate use after reconstitution is highly recommended.

● Important Note

This product is for research use only and not intended for therapeutic or in vivo diagnostic use.

● General guidelines

It is recommended to reconstitute the lyophilized ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm) with sterile deionized water to a stock solution of 5 mg/mL (1.5×10^6 beads/mL) under ISO 5 clean conditions. Separate into working aliquots and store at -70°C immediately. Upon reconstitution, immediate use is recommended for best performance.

Use a magnetic separator that is suitable for your equipment and application. Allow the beads to separate for at least 1 minute before removing supernatant. The μBeads are dense and will settle very quickly. Be sure that any μBeads mixture is homogenous before use or aliquoting.

● Preparing μBeads for use

Washing the ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm) to remove trehalose from the formulation buffer before use.

1. Resuspend the Magnetic Beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Magnetic Beads to a sterile tube.
3. Add an equal volume of sterile PBS buffer, or at least 1 mL, and mix (vortex for 5 sec, or keep on a roller for at least 2 min).
4. Place the tube on a magnet for 1 min and let the beads settle before discarding the supernatant.
5. Remove the tube from the magnet and resuspend the washed VCAM-1 μBeads in the same volume of cell culture medium as the initial volume of VCAM-1 μBeads taken from the vial (Step 2).

● Inducing T cell lineage differentiation

1. Seed CD34+ CD45+ hematopoietic stem cells with suitable cell density on culture plates.
2. Add the prepared ActiveMax® VCAM-1 μBeads and Human DLL4 μBeads, premium grade (for cells, 20 μm) with optimizing quantity and ratio for co-culture with the cells.
3. After 7 days culture intervals, the cells are counted and subjected to a full media change, including re-addition of fresh ActiveMax® VCAM-1 μBeads and Human DLL4 μBeads, premium grade (for cells, 20 μm) to modulate Notch-1 signaling for another 7 days.
4. Harvest the cells for flow cytometric analysis of the expression of T-cell progenitor markers, CD7 and CD5 on day 14.

For use in vitro, ActiveMax® VCAM-1 μBeads, premium grade (for cells, 20 μm) need to be optimized by the user according to their own experiments.

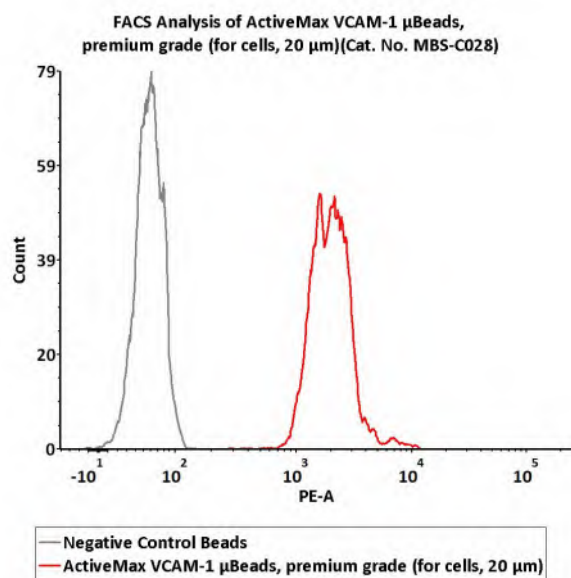
● Removing μBeads from cells

1. Transfer the culture containing cells and VCAM-1 μBeads, premium grade (for cells, 20 μm) to tube(s) and place on a magnetic for 5 min.
2. Transfer the supernatant to a new tube(s) and repeat step 1.
3. Transfer the supernatant containing the cells to a new tube for use.

● Contact Information

If you have any questions, please contact our technical support team at: TechSupport@acrobiosystems.com

- **Conjugated human VCAM-1 analyzed by FACS**



Assay of proteins on Human VCAM-1 μ Beads, premium grade (for cells, 20 μ m) surface by Flow cytometry.

The human VCAM-1 protein conjugated on the μ Beads surface were fluorescently stained using PE anti-CD106 antibody respectively, and then analyzed by flow cytometry (QC tested).