

## ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254) μBeads, premium grade (for cells)

Cat. No. MBS-C012

### ● Product Information

Product	Size	Amount
ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254) μBeads, premium grade (for cells)	2.5 mg	$2.5 \times 10^7$ beads
	10 mg (2.5 mg × 4)	$1.0 \times 10^8$ beads

### ● Product Description

ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254) μBeads, premium grade (for cells) are uniform, superparamagnetic beads of 5.5 μm with streptavidin on its surface and coupled with biotinylated Human 4-1BB Ligand / TNFSF9 (71-254) Protein, expressed from human 293 cells (HEK293).

ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254) μBeads, premium grade (for cells) 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® Human 4-1BB Ligand / TNFSF9 (71-254) μBeads, premium grade (for cells) are designed to stimulate *in vitro* 4-1BB Ligand / TNFSF9 (71-254)-specific CAR-T cells or UCAR-T cells, similar to the tumor cell lines that express human 4-1BB Ligand / TNFSF9 (71-254) antigen. It can be used as follows:

- Evaluating the characteristics of CAR-T cells or UCAR-T cells.
- *In vitro* expansion of DLL4-specific CAR-T cells or UCAR-T cells.
- *In vitro* enrichment of DLL4-specific CAR-T cells or UCAR-T cells.

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

*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.

## ● General guidelines

It is recommended to reconstitute the lyophilized ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254)  $\mu$ Beads, premium grade (for cells) with sterile deionized water to a stock solution of 5 mg/mL ( $5 \times 10^7$  beads/mL) under ISO 5 clean conditions. Separate into working aliquots and store at  $-70^{\circ}\text{C}$  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  $\mu$ Beads are dense and will settle very quickly. Be sure that any  $\mu$ Beads mixture is homogenous before use or aliquoting.

## ● Preparing $\mu$ Beads for use

Washing the ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254)  $\mu$ Beads, premium grade (for cells) 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  $\mu$ Beads in the same volume of desired cell culture medium as the initial volume of added  $\mu$ Beads in **step 2**.

## ● *In vitro* assay use

1. Activation assays: The  $\mu$ Beads are used as 4-1BB Ligand / TNFSF9 (71-254)-target cells, and co-cultured with 4-1BB Ligand / TNFSF9 (71-254)-specific CAR-T cells or UCAR-T cells at an optimized  $\mu$ Bead:cell ratio. The activation markers CD25 and CD69 should be analyzed by Flow cytometry after co-culture to monitor activation efficacy.
2. Expansion assays: The 4-1BB Ligand / TNFSF9 (71-254)-specific CAR-T cells or UCAR-T cells are seeded in the culture plates before adding  $\mu$ Beads in a series of serial  $\mu$ Bead:cell ratios. After co-incubation, the cells can be harvested for cell expansion assays.
3. Cytokine release assays: The 4-1BB Ligand / TNFSF9 (71-254)-specific CAR-T cells or UCAR-T cells are co-cultured with the  $\mu$ Beads with a series of serial  $\mu$ Bead:cell ratios for 24~72 hours. The supernatants can be collected and analyzed using commercial cytokines assay kits.

*For use in vitro, ActiveMax® Human 4-1BB Ligand / TNFSF9 (71-254)  $\mu$ Beads, premium grade (for cells) need to be optimized by the user according to their own experiments.*

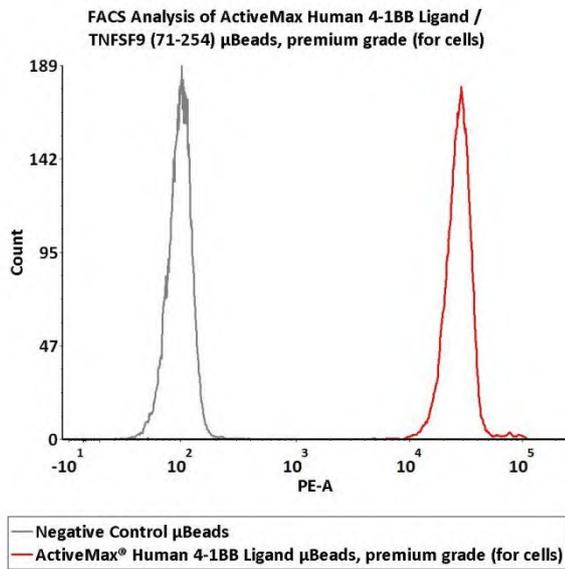
## ● Removing $\mu$ Beads from cells

1. Collect the mixture of the cells and  $\mu$ Beads after co-culturing, before centrifuging and discarding the supernatant.
2. Resuspend the pellet in suitable volume of the relevant medium, and then transfer the mixture of cells and  $\mu$ Beads into a new tube.
3. Place the tube next to a magnet for 1~2 minutes until the  $\mu$ Beads have moved to the side of the tube.
4. 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](mailto:TechSupport@acrobiosystems.com)

● **Conjugated human DLL4 analyzed by FACS**



**Assay of human 4-1BB Ligand / TNFSF9 (71-254) protein on the  $\mu$ Beads surface by Flow cytometry.** Assay of human 4-1BB Ligand / TNFSF9 (71-254) protein on the  $\mu$ Beads surface by Flow cytometry. The human 4-1BB Ligand / TNFSF9 (71-254) protein conjugated on the  $\mu$ Beads (Cat. No. MBS-C012) surface were fluorescently stained using PE anti-human CD137L (4-1BB Ligand) Antibody and analyzed by flow cytometry.