ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed

Cat. No. MBS-C001

Product Information

Product	Size	Amount
ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed	2.5 mg	2.5×10^7 beads
	10 mg	1×10^8 beads

Product Description

ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed are uniform 5.5 μm of magnetic beads coated with an optimized mixture of mouse monoclonal antibodies against the CD3 and CD28, mimicking in vivo stimulation by APCs.

ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed is intended for the in vitro isolation, stimulation and expansion of purified T cell populations of, for example, CD3+T cells, CD4+ T cells, CD8+ T cells or human PBMC in research and early preclinical stage.

Formulation

Lyophilized in PBS with 0.1% HSA, pH 7.4. Normally 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 CoA.

Storage

The product is shipped at ambient temperature. Upon receipt, store it immediately at -20°C or below for long term storage.

This product is stable after storage at:

1 -20°C or below for 5 years in lyophilized state from date of receipt;

2. 2-8°C for 12 months under sterile conditions after reconstitution.

Please avoid repeated freeze-thaw cycles after reconstitution.

Important Note

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

General guidelines

Prepare beads

Because magnetic beads are 5.5 μm particle size, the beads may stick to the side of the bottle in the shipping process. Before opening, it is recommended to gently shake the bottle to settle the beads. Avoid vigorous mixing such as vortex mixing. The beads are dense and will tend to settle very quickly. Be sure that any bead mixture is homogenous before aliquoting.

Always work under sterile conditions to avoid contamination.

It is strongly recommended to reconstitute the lyophilized ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed with sterile deionized water to a stock solution of 5 mg/mL (5 × 10^7 beads/mL), store at 2 to 8 °C.

Wash ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed 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 tube.
3. Add an equal volume of PBS buffer containing 1% HSA, or at least 1mL, and mix (vortex for 5 sec, or keep on a roller for at least 2 min).
4. Place the tube on a magnet for 2 min and then discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Magnetic Beads in the same volume of T cell culture medium as the initial volume of Magnetic Beads taken from the vial (step 2) .

Prepare cells

1. ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed is intended for the in vitro stimulation and expansion of purified T cell populations of, for example, CD3+T cells, CD4+ T cells, CD8+ T cells or human PBMC.
2. Prepare T cell culture medium, such as RPMI1640 with 10% of FBS, CTS™ OpTmizer™ T-Cell Expansion SFM (Thermoscientific-gibco), X-VIVO-15 Serum-free Hematopoietic Cell Medium (Lonza) and other T cell culture medium.

Isolate human CD3+T Cells

The isolation protocol uses a beads-to-T cells ratio of 1:1, so must take some washed cells to calculate the viability, concentration, and number of CD3+ T cells before starting cell isolation.

1. Take human PBMC from liquid nitrogen and thaw immediately at 37℃ for 5minutes.
2. Harvest the cells and wash once by PBS buffer containing 1% HSA, and count the cells number and the viability.
3. Detect the positive rate of CD3+T cells by flow cytometry, and calculate the number of CD3+T cells.
4. Adjust the cell density of 1 × 10^7 CD3+T cells with PBS buffer containing 1% HSA in 1.5mL or 4mL sterile centrifuge tube.
5. Add pre-washed and resuspended ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed to obtain a beads-to-CD3+T cells ratio of 1:1 *(The volume of cell-Beads suspension should not exceed half of the maximum volume of the centrifuge tube)*.
6. Place the tube to 3D Rotating Mixer (MIULAB, RH-18+) and mix thoroughly for 30min at RT.
7. Transfer the tube on a magnet for 2 min to separate the beads-T cells from the solution.
8. Carefully pipette (do not pour) off the supernatant to a new tube, this is the unlabeled cell fraction.
9. Remove the tube from the magnet, this tube contains the isolated beads-cells complex.
10. Resuspend the isolated complex in the tube with the fresh T cell culture medium for downstream applications.
11. The supernatant collected from *Step8* is used to calculate the isolated efficiency.

*Note： Calculation formula of isolated efficiency: The isolated efficiency of CD3+T cells=(1-the number of CD3+T cells in the non-captured cells in step 8/the total number of CD3+T cells invested in step 4)*100%, Wherein, the number of CD3+T cells = the number of cells measured by cell counter * the positive rate of CD3+T cells)*

Activate Human T Cells

The following procedure describes activation of human T cells in 12-well tissue culture plate. Activation can also be performed in other cell culture devices, but the ratio of ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed to T cells must be 1:1.

1. Start with 5× 10^6 purified T cells in 0.5 mL of T cell culture medium in a 12-well tissue culture plate.
2. Add 0.5 mL pre-washed and resuspended ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed (2.5 × 10^6 beads) to obtain a beads-to-cells ratio of 1:1.
3. Incubate in a humidified CO_2 incubator at 37 °C, according to your specific experimental requirements (Incubation time is

recommended for 24 hours).

4. Harvest the activated T cells and use directly for further analysis.

5. For flow cytometry applications, remove the beads prior to staining. Place the tube on a magnet for 2 min to separate the beads from the solution. Transfer the supernatant containing the cells to a new tube.

Expand Human T Cells

1. Prepare T cell culture medium supplemented with 4ng/mL recombinant human IL-2 (rhIL-2).
2. Start with 1–1.5 × 10^6 purified T cells/mL in T cell culture medium in a suitable tissue culture plate or tissue culture flask.
3. Add ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade DMF Filed at a beads-to-cells ratio of 1:1.
4. Incubate in a humidified CO_2 incubator at 37°C, according to your specific experimental requirements.
5. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate are typically observed in exhausted cell cultures.
6. When the cell density exceeds 2.0-2.5 × 10^6 cells/mL or when the medium turns yellow, split cultures back to a density of 4–8 × 10^5 cells/mL with the complete T cell culture medium.

Typical Data

Activation human T cells analyzed by FACS

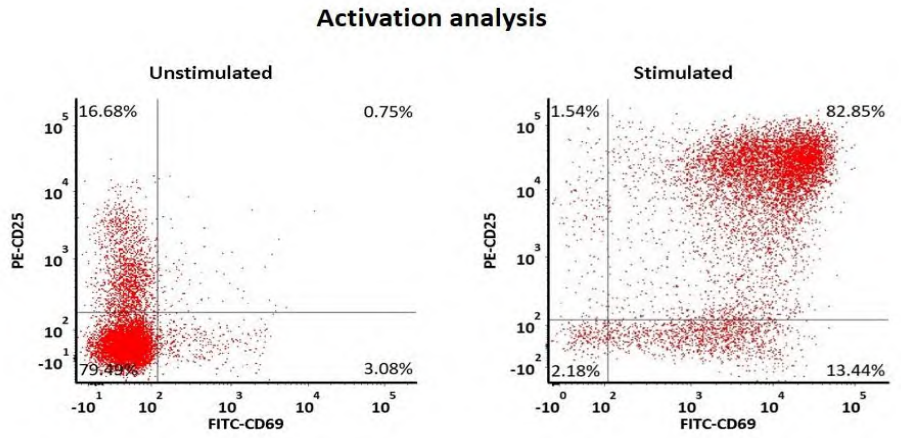


Fig.1. Activation of the purified human T Cells. The human T cells were stimulated with ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade(Cat. No. MBS-C001) for 24hrs, and the activation was assessed by measuring expression of both activation markers CD25 and CD69 expression on the T cells surface by stanning with PE labeled anti-human CD25 antibody and FITC labeled anti-human CD69 antibody respectively (QC tested).

Activation of the human T Cells expansion

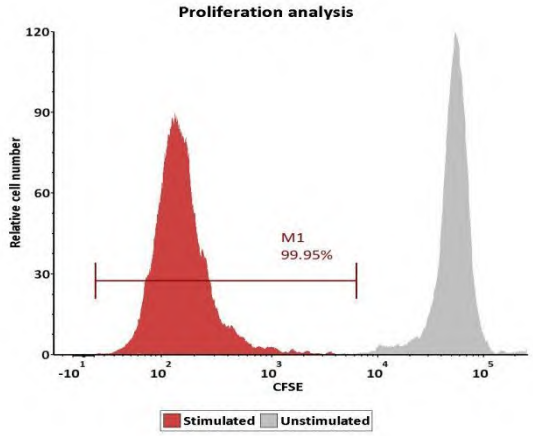
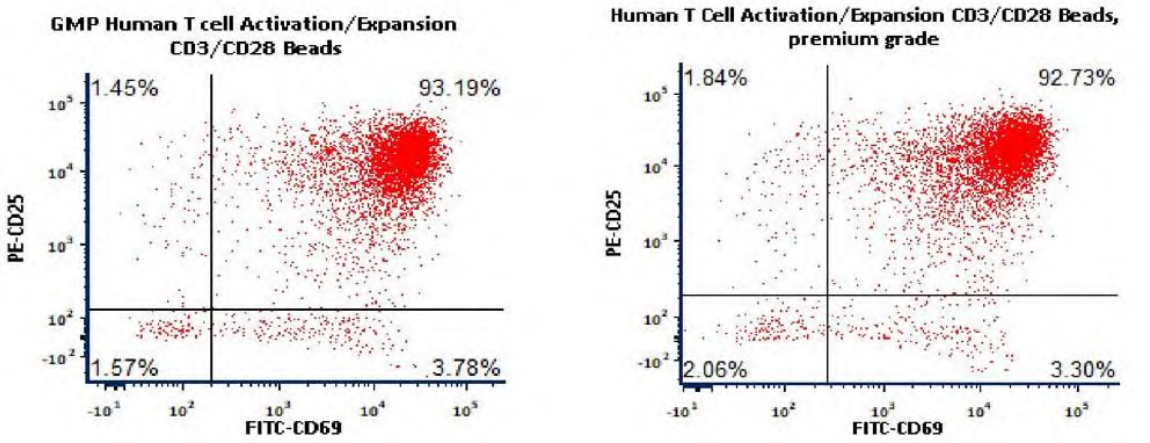
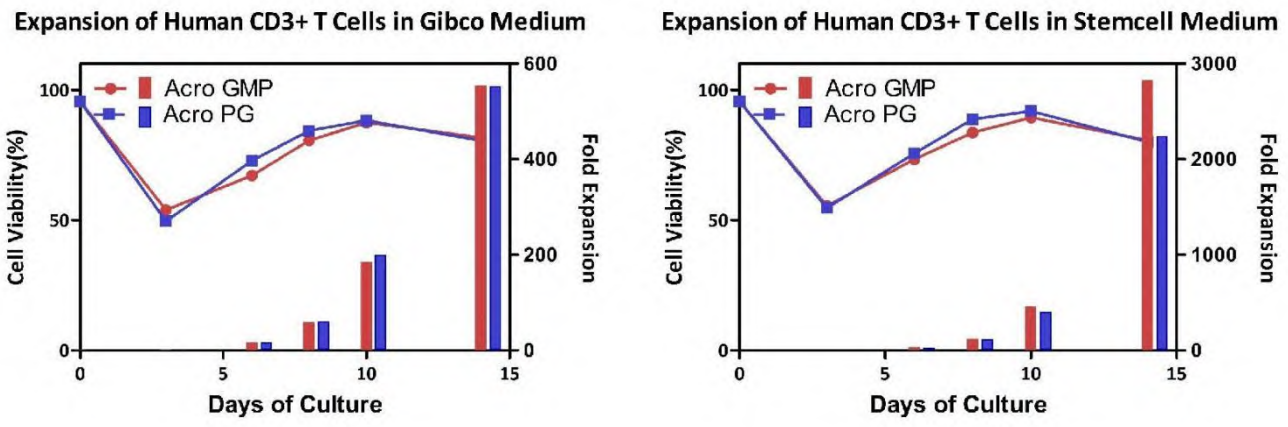


Fig.2. Purified human T Cells expansion. The human T cells were labeled with carboxy fluorescein succinimidyl ester (CFSE) and stimulated with ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, premium grade(Cat. No. MBS-C001), and then the proliferation of the T cells was assessed with CFSE dilution assay by flow cytometry on day 5 after stimulation (QC tested).

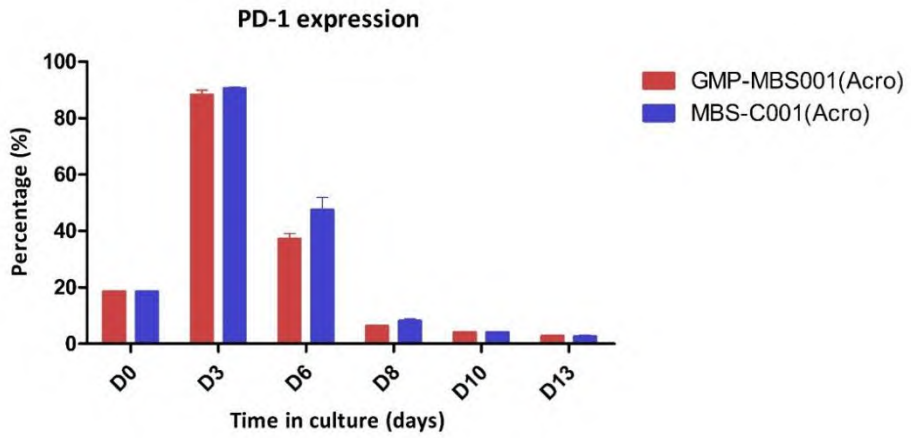
- **Stability**



Activation of the purified human T Cells. The purified human T cells were activated using Human T cell Activation/Expansion CD3/CD28 Beads, (ACRO, Cat. No. GMP-MBS001/MBS-C001) respectively for 24 hours with CTS Optimizer Medium. Cells were fluorescently stained using PE labeled anti-human CD25 antibody and labeled FITC anti-human CD69 antibody and analyzed by flow cytometry.



Expansion of the human CD3+T cells. Human T cells using ACROBiosystems CD3/CD28 Beads (ACRO, Cat. No. GMP-MBS001/MBS-C001) were expanded under two different medium, respectively. Expansion was performed for two weeks, showing that ACROBiosystems' GMP and PG beads showing similar proliferative abilities.



PD-1 expression of the activated human T Cells. The purified human T cells were stimulated using Human T cell Activation/Expansion CD3/CD28 Beads at a ratio of 1:1 beads-to-cells. Cells were expanded in T cell culture medium supplemented with 4ng/mL of rhIL-2 Protein. Activated T cells were expanded for up to 8 days with low PD-1 expression.

Contact Information

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