GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads

Cat. No. GMP-MBS001

Product Information

Product	Size	Amount
GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads	10 mg	1×10^8 beads
	100 mg(10 mg × 10)	1×10^9 beads
	400 mg(10 mg × 40)	4×10^9 beads

ACROBiosystems provide rigorous quality control tests (fully validated equipment, processes and test methods) on our GMP grade products to ensure that they meet stringent standards in terms of safety, activity and inter-batch stability, and each bulk QC lot mainly contains the following specific information:

Test items	Method	Specification
Physical appearance	Visual method	Brown liquid
Labelled quantity	Volumetric method	Not less than labelled quantity
Particle counting test	Counting using Cell counter	5.00×10^7 beads/mL ± 10%
Antibody leakage assay	ELISA	<0.50 μg/mL
T cell activation assay	FACS	> 80% CD25/CD69 coexpression
T cell proliferation assay	CFSE stanning	> 80% of proliferated T cells
Endotoxin	LAL method	<0.5 EU/mL
Sterility <USP 71>	Membrane filtration method	Negative

Features

1. Designed in ISO 9001:2015 and ISO 13485:2016 certified facility
2. Manufactured and QC tested under a GMP compliance factory
3. Animal-Free materials
4. GMP grade antibodies as raw materials with strict virus removal steps and testing
5. GMP grade recombinant HSA as excipient
6. Batch-to-batch consistency
7. Stringent quality control tests

Product Description

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

GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads are 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 for cell based clinical and preclinical research.

GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads are Manufactured under a GMP compliance factory with animal free raw materials, and tested under GMP guidelines.

Formulation

GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads is supplied with $5x10^7$ beads/mL in PBS, pH 7.4, with 0.1% recombinant human serum albumin (recombinant HSA).

Shipping and Storage

This product is supplied and shipped as sterile liquid solution with blue ice, please inquire the shipping cost.

The product MUST be stored at 2-8°C upon receipt;

This product is stable after storage at 2-8°C for 5 years under sterile conditions.

General guidelines

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.

Prepare beads

Wash GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads 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. GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads 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 GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads 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

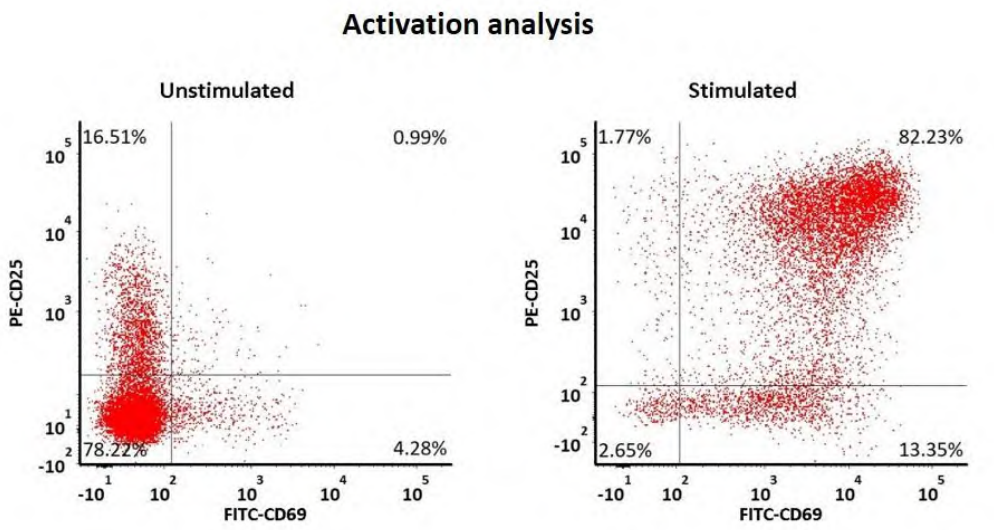
The following procedure describes activation of human T cells in 12-well tissue culture plate. Activation can also be performed in other cell culture devices, but the ratio of GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads to T cells must be 1:1.

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 GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads (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.

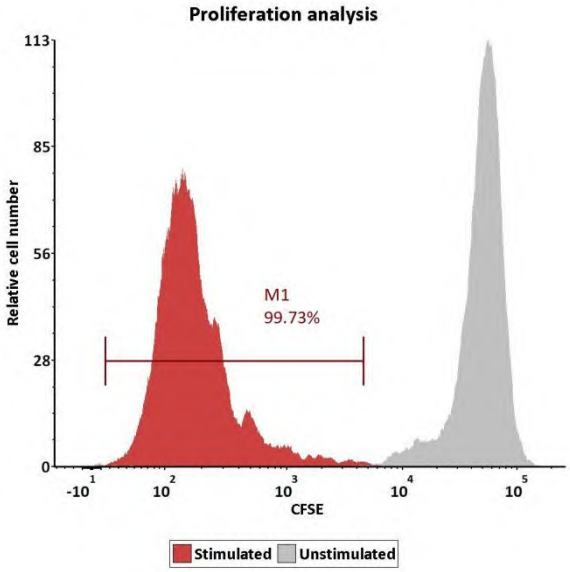
Expand Human T Cells

1. Prepare T cell culture medium supplemented with 4ng/mL recombinant human IL-2 (rhIL-2) (Acrobiosystems, Cat. No. IL2-H4113).
2. Start with $1–1.5 \times 10^6$ purified T cells/mL in T cell culture medium in a suitable tissue culture plate or tissue culture flask.
3. Add GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads 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 $2.0\text{-}2.5 \times 10^6$ cells/mL or when the medium turns yellow, split cultures back to a density of $4–8 \times 10^5$ cells/mL with the complete T cell culture medium.

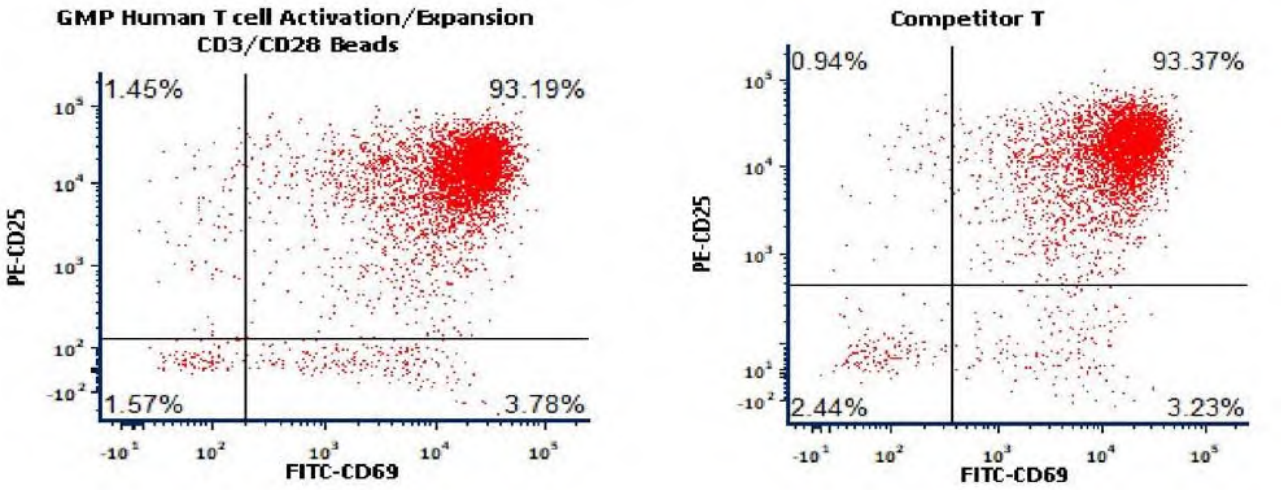
- **Typical Data**



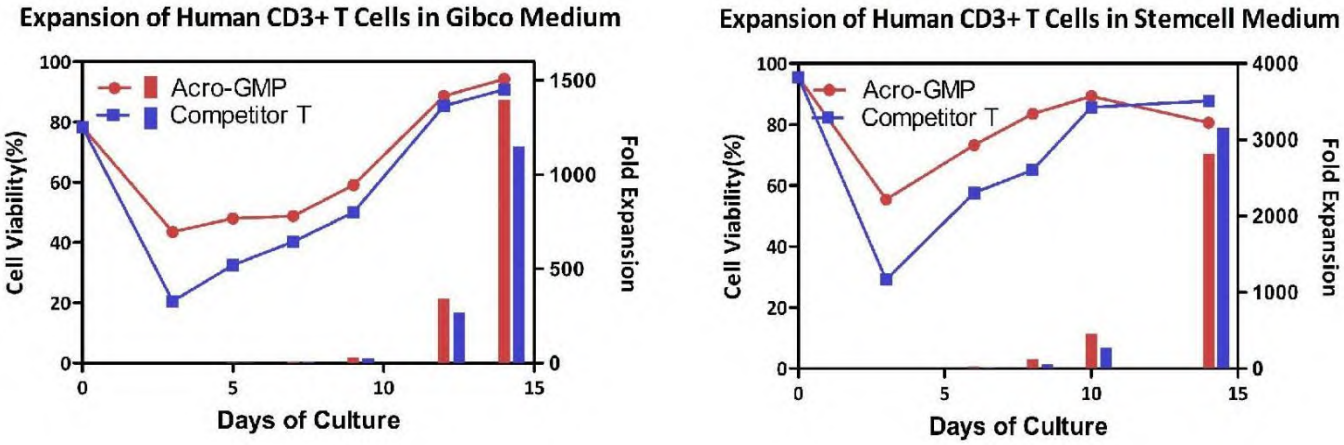
The human T cells were stimulated with GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads (Cat. No. GMP-MBS001) for 24hrs, and the activation was assessed by measuring expression of both activation markers CD25 and CD69 expression on the T cells surface by stanning with PE labeled anti-human CD25 antibody and FITC labeled anti-human CD69 antibody respectively (QC tested).



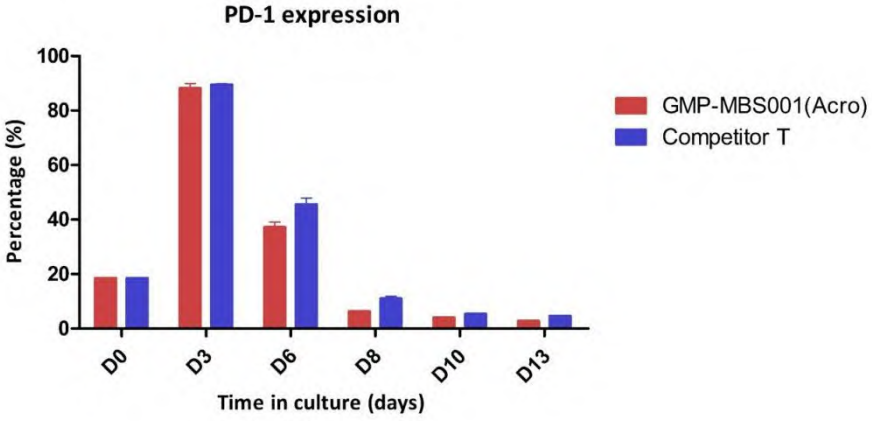
The human T cells were labeled with carboxy fluorescein succinimidyl ester (CFSE) and stimulated with GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads (Cat. No. GMP-MBS001), and then the proliferation of the T cells was assessed with CFSE dilution assay by flow Cytometry on day 5 after stimulation (QC tested).



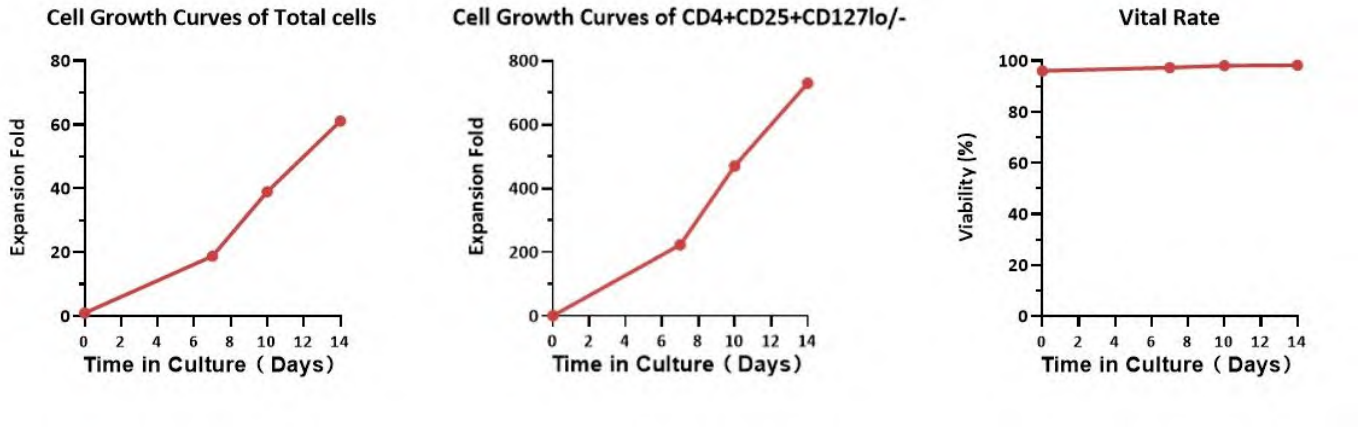
Activation of the purified human T Cells. The purified human T cells were activated using Human T cell Activation/Expansion CD3/CD28 Beads (ACRO, Cat. No. GMP-MBS001) and Competitor-Beads respectively for 24 hours with CTS Optimizer Medium. Cells were fluorescently stained using PE labeled anti-human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



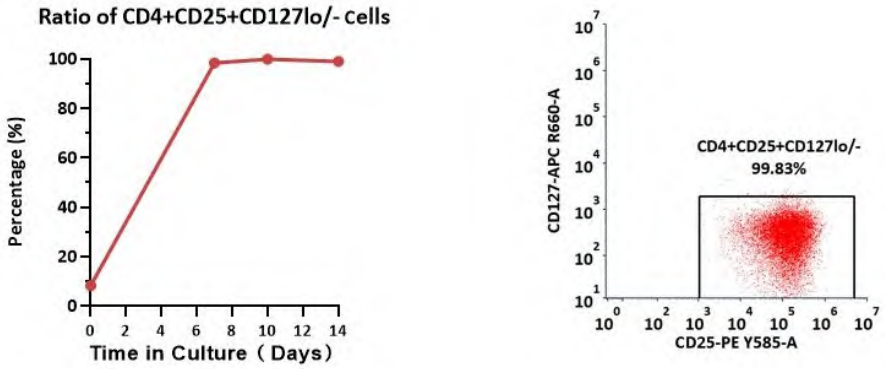
Expansion of the human CD3+T cells. Human T cells using ACROBiosystems CD3/CD28 Beads (ACRO, Cat. No. GMP-MBS001) were expanded under two different medium, respectively. Expansion was performed for two weeks, showing that ACROBiosystems' beads showing better proliferative abilities and comparable competitive ideas compared with competitor product.



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 (Acrobiosystems, Cat. No. IL2-H4113). Activated T cells were expanded for up to 8 days with low PD-1 expression.

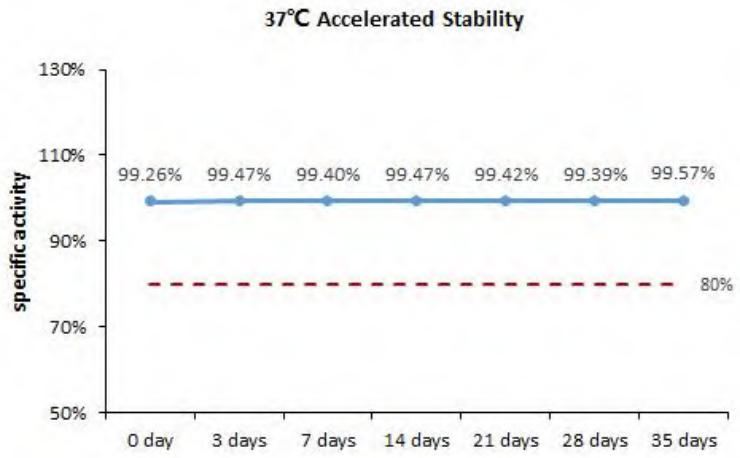


Human CD4+ cells were activated with GMP ActiveMax Human T cell Activation/Expansion CD3/CD28 Beads (Cat. No. GMP-MBS001), and cultured with GMP Human IL-2 Protein (Cat. No. GMP-L02H14), GMP Human TGF-Beta 1 Protein (Cat. No. GMP-TG1H25), Rapamycin, all-trans retinoic acid and sodium butyrate in CelThrea™ GMP T Cell Expansion Medium (Cat. No. GMP-CM3101) for two weeks. The result shows that CelThrea™ GMP T Cell Expansion Medium with GMP ActiveMax Human T cell Activation/Expansion CD3/CD28 Beads, GMP Human IL-2 Protein and GMP Human TGF-Beta 1 Protein can promote the expansion of Treg cells with a reasonable cell viability.

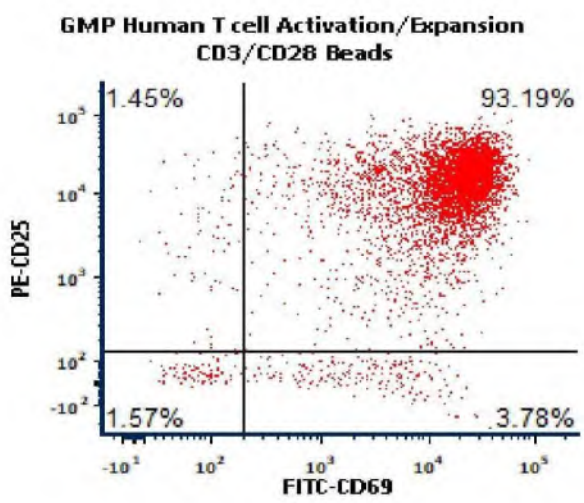


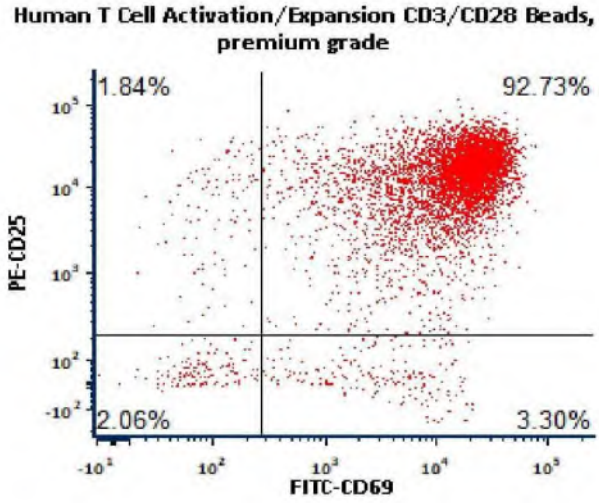
Human CD4+ cells were activated with GMP ActiveMax Human T cell Activation/Expansion CD3/CD28 Beads (Cat. No. GMP-MBS001), and cultured with GMP Human IL-2 Protein (Cat. No. GMP-L02H14), GMP Human TGF-Beta 1 Protein (Cat. No. GMP-TG1H25), Rapamycin, all-trans retinoic acid and sodium butyrate in CelThrea™ GMP T Cell Expansion Medium (Cat. No. GMP-CM3101) for two weeks. The result shows that CelThrea™ GMP T Cell Expansion Medium with GMP ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads, GMP Human IL-2 Protein and GMP Human TGF-Beta 1 Protein can increase the percentage of the CD4+CD25+CD127lo/-. With the post-culture time, the proportion of CD4+CD25+CD127lo/- cells remained unchanged.

- **Stability**



The Cell based assay shows that GMP ActiveMax® Human T cell Expansion CD3/CD28 Beads(Cat. No. GMP-MBS001) is stable at 37℃ for 35 days.





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.

Contact Information

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

MANUFACTURING SPECIFICATIONS

ACROBiosystems GMP grade products are produced under a quality management system and in compliance with relevant guidelines: Ph. Eur General Chapter 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products; USP<92>Growth Factors and Cytokines Used in Cell Therapy Manufacturing; USP<1043>Ancillary Materials for Cell, Gene, and Tissue-Engineered Products; ISO/TS 20399-1:2018, Biotechnology - Ancillary Materials Present During the Production of Cellular Therapeutic Products.

ACROBiosystems Quality Management System Contents:

1. Designed in ISO 9001:2015 and ISO 13485:2016 certified facility, Manufactured and QC tested under a GMP compliance factory
2. Animal-Free materials
3. Materials purchased from the approved suppliers by QA
4. Qualified personnel
5. Quality-related documents review and approve by QA
6. Fully batch production and control records
7. Equipment maintenance and calibration
8. Validation of analytical procedures
9. Stability studies conducted
10. Comprehensive regulatory support files

Request For Regulatory Support Files（RSF）

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