

# **GMP Cloudz™ Human T Cell Activation Kit**

Catalog Number CLD001-GMP

This package insert must be read in its entirety before using this product.  
For Preclinical or *Ex Vivo* Clinical Use Only.  
Not for use in Diagnostic Procedures.

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## DESCRIPTION

T Lymphocytes (T Cells) are a critical component of the adaptive immune response. While dysregulation of T cell function and proliferation contributes to the etiology of many diseases, the ability of T cells to activate an immune response toward specific antigens is being harnessed as a powerful immunotherapy tool to combat cancer and other diseases (1). To facilitate discovery and preclinical research for T cell immunoregulation and therapy, robust platforms for ex vivo expansion and maintenance of T cells, including non-magnetic based GMP-grade activation particles.

The GMP Cloudz™ Human T Cell Activation Kit is designed to activate and expand human T cells from peripheral blood mononuclear cells (PBMCs) or from enriched T cell populations. This kit is manufactured following cGMP practices and contains GMP-grade components that are optimized for incorporation into cell manufacturing workflows.

The GMP Cloudz Human T Cell Activation Kit contains GMP-grade Cloudz CD3/CD28 and GMP-grade Cloudz 6X Release Buffer. Cloudz CD3/CD28 are dissolvable microspheres functionalized with recombinant human anti-CD3 and CD28 antibodies. When used in combination with recombinant human GMP cytokines and T cell expansion media, Cloudz CD3/CD28 induce robust expansion of T cells in culture. The Cloudz 6X Release Buffer is designed to minimize post-expansion cell processing steps and can be added directly into the cell culture vessel where it will rapidly dissolve Cloudz CD3/CD28.

Each kit contains enough reagents to activate  $4 \times 10^8$  CD3<sup>+</sup> T cells. Fold expansion rates may vary based on individual protocols, reagent combinations, and variability in donor cell populations.

## LIMITATIONS

- This reagent should not be used beyond the expiration date indicated on the label.
- Do not use if package is damaged. Use undamaged and sealed bottles only.
- Results may vary due to variations among cells derived from different donors.

## PRECAUTIONS

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	AMOUNT PROVIDED	STORAGE OF OPENED/RECONSTITUTED MATERIAL
GMP-grade Cloudz CD3/CD28	SPC-4205	2 x 12 mL	Store at 2-8 °C.*
GMP-grade 6X Release Buffer	SPC-4185	2 x 100 mL	

\* Provided this is within the expiration date of the kit.

## OTHER MATERIALS REQUIRED

- Fresh or cryopreserved human peripheral blood mononuclear cells (PBMCs) or CD3<sup>+</sup> T lymphocytes obtained from human peripheral blood or leukapheresis
- ExCellerate™ Human T Cell Expansion Media (R&D Systems, Catalog # CCM030)
- Recombinant Human IL-2 GMP Protein (R&D Systems, Catalog # 202-GMP)
- Culture Vessel (e.g. plates, culture flasks, culture bags)
- Sterile 1X PBS
- Cell counter

## REAGENT PREPARATION

Reagents in the GMP Human Cloudz T Cell Activation Kit are supplied in septum-cap bottles for incorporation into cell manufacturing work flows.

**GMP-grade Cloudz CD3/CD28** - GMP-grade Cloudz CD3/CD28 are supplied as a ready-to-use reagent.

**Note:** *We recommend using the Vialok<sup>®</sup> Vented Vial Access Device (Fresenius Kabi, Catalog # YM020) to access GMP-grade Cloudz CD3/CD28. These needle-free adapters can be attached to the vials allowing the reagent to be removed via a luer-lock syringe).*

**GMP-grade 6X Release Buffer** - GMP 6X Release Buffer is a ready-to-use concentrate that can be added directly into cell culture media at a 1:6 dilution (*i.e.*, add 100 mL of GMP 6X Release Buffer directly into a cell culture vessel containing 500 mL of culture medium).

**1X Release Buffer** - If a 1X Release Buffer is desired, add 20 mL of GMP-grade 6X Release Buffer to 100 mL of Sterile 1X PBS in an appropriate reagent vessel.

## PROTOCOL FOR HUMAN T CELL EXPANSION

Each vial contains a minimum of 12 mL of GMP-grade Cloudz CD3/CD28, which is sufficient to activate and expand up to 240 million starting cells. We recommend using 50 µL of GMP-grade Cloudz CD3/CD28 for every 1 million starting cells.

### CELL CULTURE SETUP AND ACTIVATION

1. Prepare starting cells by thawing/washing according to desired protocol.
2. Count cells.
3. Resuspend starting cells in ExCellerate Human T Cell Expansion Media containing 20 ng/mL of IL-2 and transfer into desired culture vessel.
4. Mix GMP-grade Cloudz CD3/CD28 by vortexing the vial for 5-10 seconds immediately before use.
5. Add 50 µL of GMP-grade Cloudz CD3/CD28 for every 1 million starting cells.
6. Gently mix cells with GMP-grade Cloudz CD3/CD28 by shaking or rocking for approximately 30 seconds.
7. Culture cells in a humidified incubator (37°C, 5% CO<sub>2</sub>) for 9 days.

**Note:** *Monitoring T cell expansion may be desired. Every 2-3 days retrieve a sample of your cell culture suspension and count the cells. For accurate cell counts, it is highly recommended to dilute the cells in 1X Release Buffer prior to counting. Based on cell counts, add additional Complete Cell Culture Medium to each culture vessel to maintain your preferred cell density of choice (~ 5 x 10<sup>5</sup> cells/mL is recommended). If required, transfer cell suspensions to larger culture vessels. Culture cells in humidified incubator (37°C, 5% CO<sub>2</sub>) and continue to monitor cell density until the desired number of fold-expansion is achieved.*

### DISSOLUTION OF GMP-GRADE CLOUDZ CD3/CD28 AND CELL COLLECTION

#### **Protocol 1 - Direct Addition of GMP-grade 6X Release Buffer into Cell Culture Medium.**

GMP-grade 6X Release Buffer is a ready-to-use concentrate that can be added directly into cell culture media at a 1:6 dilution (*i.e.*, add 100 mL of GMP-grade 6X Release Buffer directly into a cell culture vessel containing 500 mL of culture medium). After addition of the buffer, agitate the sample to ensure GMP-grade Cloudz CD3/CD28 is completely dissolved (approximately ~5 minutes). Collect T cells by centrifugation for downstream applications.

## Protocol 2 – Dissolution Using 1X Release Buffer

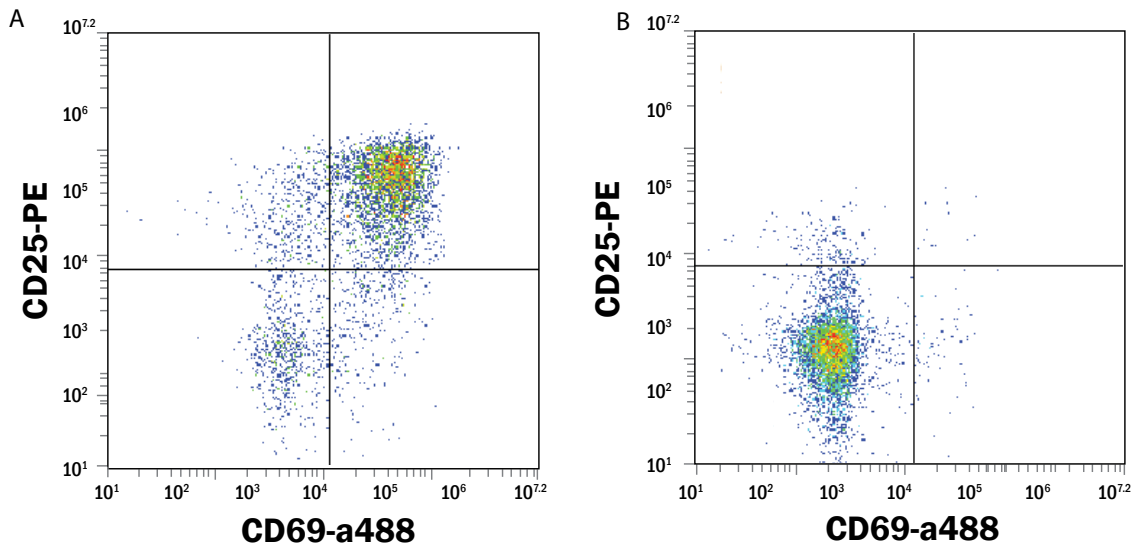
1. Prepare 1X Release Buffer as indicated in Reagent Preparation Section.
2. Transfer T cell suspension (still containing GMP-grade Cloudz CD3/CD28) into sterile centrifuge tubes. Centrifuge at 300 x g for 5 minutes at room temperature.
3. Remove the supernatant. Re-suspend the cell pellet based on the following recommendations:

AMOUNT OF GMP-GRADE CLOUDZ CD3/CD28 PARTICLES ADDED TO EACH SAMPLE ON DAY 0	VOLUME OF 1X RELEASE BUFFER
25 µL - 100 µL	2 mL
100 µL - 400 µL	4 mL
400 µL - 2 mL	8 mL

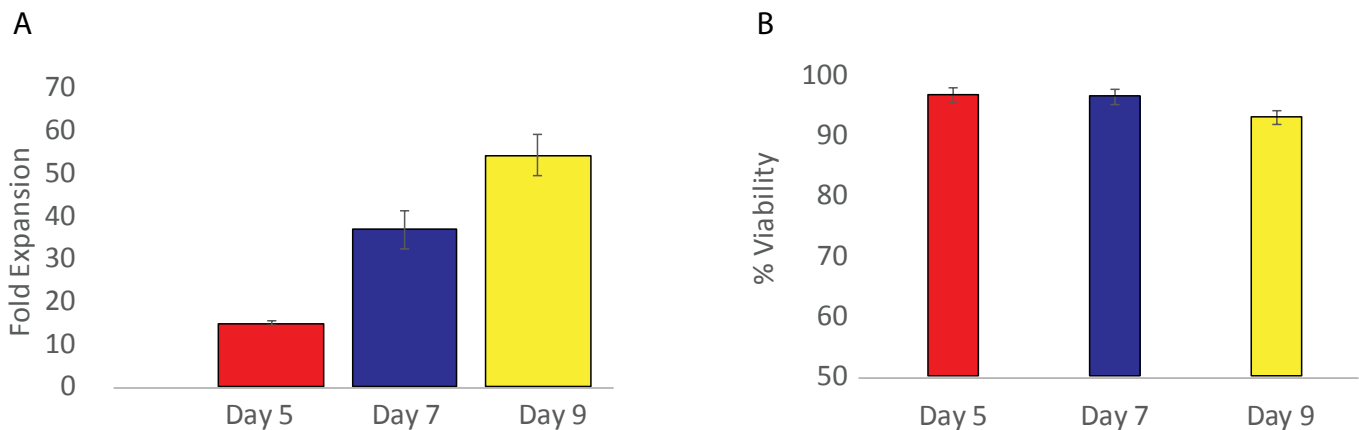
**Note:** For larger volumes of GMP-grade Cloudz CD3/CD28 particles in each sample, scale up volume of 1X GMP Release Buffer as needed.

4. Agitate this suspension to dissolve GMP-grade Cloudz CD3/CD28 by pipetting (up and down 5 times), rocking, or shaking for no more than 5 minutes.
5. Centrifuge the cell suspension at 300 x g for 5 minutes at room temperature.
6. Remove the supernatant. Re-suspend the cell pellet in appropriate media or buffer for downstream analysis or applications.

## DATA EXAMPLES



**Example of T cell Activation. A.** Primary human CD3<sup>+</sup> cells were activated with GMP Cloudz™ Human T Cell Activation Kit and cultured for 48 hours in ExCellerate™ T Cell Expansion Media (R&D Systems, Catalog # CCM030) and 20 ng/mL IL-2 (R&D Systems, Catalog # 202-GMP). **B.** The negative control was performed without adding the Cloudz T-cell reagent. Cells were fluorescently stained with using CD25-PE (R&D Systems, Catalog # FAB1020P) and CD69-a488 (R&D Systems, Catalog # FAB23591G) and analyzed via flow cytometry. Dead cells and debris were excluded from the analysis based on scatter signals and viability dye. Cells that were treated with GMP Cloudz™ Human T Cell Activation Kit showed an activation signal 48 hours after reagent addition.



**Expansion of Human T Cells using Cloudz™ Human T Cell Activation Kit.** Primary human CD3<sup>+</sup> cells were activated with GMP Cloudz™ Human T-Cell Activation Kit and cultured for 9 days in ExCellerate™ T Cell Expansion Media (R&D Systems, Catalog # CCM030) and 20 ng/mL IL-2 (R&D Systems, Catalog # 202-GMP). **A.** Cell counts were performed to determine fold expansion compared to the Day 0 seeding density ( $0.25 \times 10^6$  cells/mL). **B.** Percent Viability was determined on days 5, 7, and 9 of the experiment.



## REFERENCES

1. June, C.H. *et al.* (2018) *Science* **359**:136.

## MANUFACTURING SPECIFICATIONS

R&D Systems® GMP-grade products are produced according to relevant sections of the following documents: WHO TRS, No. 822,1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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