

Cloudz™ Human T Cell Activation Kit

Catalog Number CLD001

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
DESCRIPTION	1
LIMITATIONS.....	1
PRECAUTIONS.....	1
MATERIALS PROVIDED & STORAGE CONDITIONS	2
OTHER MATERIALS REQUIRED	2
REAGENT PREPARATION.....	2
PROTOCOL FOR HUMAN T CELL EXPANSION	3
DATA EXAMPLES	5
REFERENCES	5

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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 activation particles.

The 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.

The Cloudz Human T Cell Activation Kit contains Cloudz CD3/CD28 and 6X Release Buffer. Cloudz CD3/CD28 are dissolvable microspheres functionalized with recombinant human anti-CD3 and anti-CD28 antibodies. When used in combination with recombinant human cytokines and T cell expansion media, Cloudz CD3/CD28 induce robust expansion of T cells in culture. The 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 1×10^6 CD3⁺ T cells, resulting in approximately 50-fold expansion in 9 days. Fold expansion rates may vary based on individual protocols, reagent combinations, and variability in donor cell populations.

LIMITATIONS

- The most recent version of the End User Terms of Use of Product may be found at: RnDSystems.com/Legal-information.
- 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.
- For research use only.
- Not intended for direct administration into humans or animals. Not for parenteral use.

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
Cloudz CD3/CD28	SPC-4263	12 mL	Store at 2-8 °C.*
6X Release Buffer	SPC-4262	100 mL	

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

OTHER MATERIALS REQUIRED

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

REAGENT PREPARATION

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

6X Release Buffer - 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 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 6X Release Buffer to 100 mL of Sterile 1X PBS in an appropriate reagent vessel.

PROTOCOL FOR HUMAN T CELL EXPANSION

A vial of Cloudz CD3/CD28 contains a minimum of 12 mL, which is sufficient to activate and expand up to 240 million starting cells. For expansion from PBMCs, we recommend 20 μ L for every 1 million PBMCs. If starting from a purified population of CD3⁺ cells, we recommend adding 50 μ L for every 1 x 10⁶ 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 Cloudz CD3/CD28 by vortexing the vial for 5-10 seconds immediately before use.
5. Add 50 μ L of Cloudz CD3/CD28 for every 1 x 10⁶ starting cells. Add the appropriate amount of Cloudz CD3/CD28 particles based on the recommendations stated above (20 μ L Cloudz CD3/CD28 for every 1 x 10⁶ PBMCs or 50 μ L Cloudz CD3/CD28 for every 1 x 10⁶ CD3⁺ cells).
6. Gently mix cells with Cloudz CD3/CD28 by shaking or rocking for approximately 30 seconds.
7. Culture cells in a humidified incubator (37 °C, 5% CO₂) 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 10⁵ cells/mL is recommended). If required, transfer cell suspensions to larger culture vessels. Culture cells in humidified incubator (37 °C, 5% CO₂) and continue to monitor cell density until the desired number of fold-expansion is achieved.*

DISSOLUTION OF CLOUDZ CD3/CD28 AND CELL COLLECTION

Protocol 1 – Direct addition of 6X Release Buffer into Cell Culture Medium

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 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 Cloudz CD3/CD28 is completely dissolved (approximately 5 minutes). Collect T cells by centrifugation for downstream applications.

PROTOCOL FOR HUMAN T CELL EXPANSION *CONTINUED*

Protocol 2 – Dissolution Using 1X Release Buffer

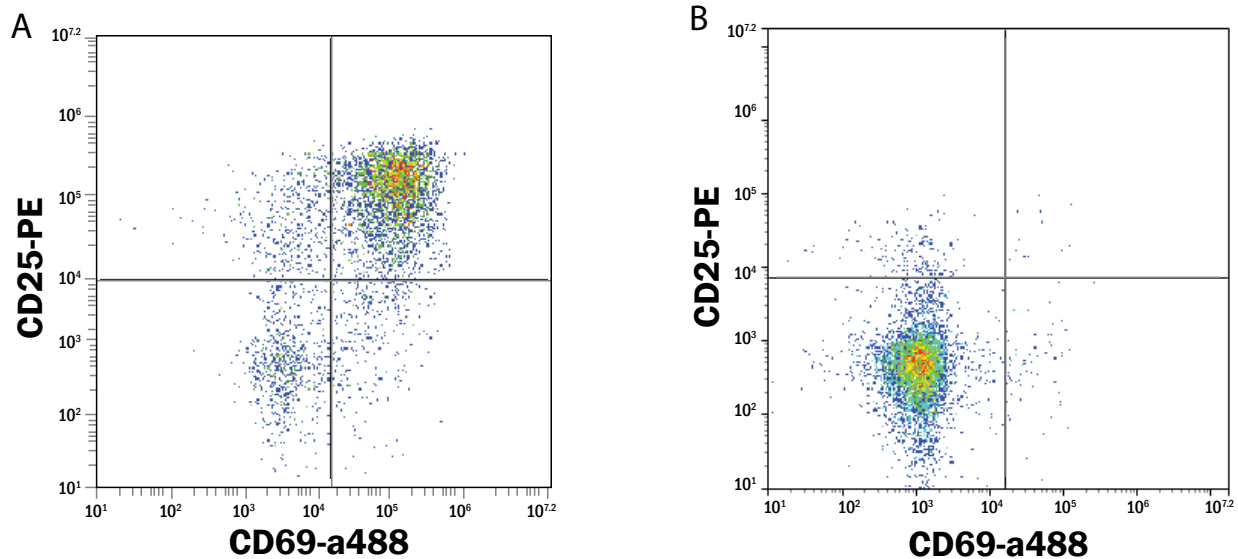
1. Prepare 1X Release Buffer as indicated in Reagent Preparation Section.
2. Transfer T cell suspension (still containing Cloudz CD3/CD28) into sterile centrifuge tubes. Centrifuge at 300 x g for 5 minutes at room temperature.
3. Remove the supernatant. Re-suspend the cell pellet based on the following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

AMOUNT OF 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

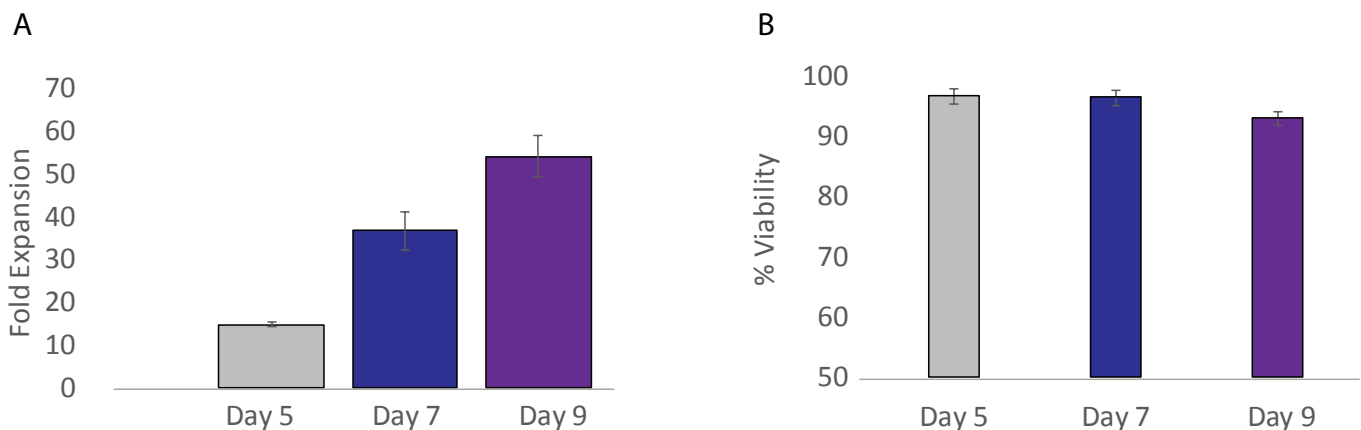
Note: For larger volumes of Cloudz CD3/CD28 particles in each sample, scale up volume of 1X Release Buffer as needed.

4. Agitate this suspension to dissolve 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⁺ cells were activated with 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-IL). **B.** The negative control was performed without adding the Cloudz CD3/CD28. 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 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⁺ cells were activated with 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 x 10⁶ 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**:1361.

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