Description

Human T Cell Activation Reagent is a ready-to-use reagent designed for *in vitro* activation and expansion of human T cells. This reagent consists of soluble CD3 and CD28 antibody complexes that can bind to CD3 and CD28 on the surface of T cells, providing primary and co-stimulatory signals for T cell activation.

Background

T cells are important immune cells that have a variety of functions. T cells can eliminate infected or cancerous cells and direct the immune response by helping B lymphocytes eliminate invading pathogens. T cells play a critical role in destroying cancer cells by recognizing antigens presented on cancer cells. CAR (chimeric antigen receptor)-T cell therapy has proven to be effective against some types of cancer, even when other treatments are not effective. T cells in general are widely used in cancer and research of other human diseases. However, T cells require activation to become active and proliferate. *In vitro* activation typically involves the use of CD3 and CD28 antibodies, in multiple forms, to engage the T cell receptor and use a co-stimulatory protein, attempting to mimic the situation *in vivo* where T cells are activated by the MHC (major histocompatibility complex) and APC (antigen presenting cells). This process of activation and expansion is crucial to generate enough cells for development and clinical applications.

Application(s)

T cell activation and expansion for downstream applications, such as cytotoxicity assays, CAR (chimeric antigen receptor)-T cell development and flow cytometry.

Supplied Materials

This kit is provided with enough reagents and materials for the activation of 1 x 10^9 T cells in 1000 ml of T cell expansion media, in one reaction or multiple reactions of smaller numbers of T cells.

Materials Required but Not Supplied

Name	Ordering Information
Normal Human Peripheral Blood Mononuclear Cells, Frozen	BPS Bioscience #79059
Human T Cell Isolation Kit	BPS Bioscience #82288
Thaw Medium 2	BPS Bioscience #60184
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	BPS Bioscience #90184

Storage Conditions

This assay kit will perform optimally for up to **6 months** from the date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product contains small amounts of sodium azide. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

General Notes

- The following protocol was designed for the activation of 1 x 10^7 T cells in 10 ml of T cell expansion media. To perform activation of different cells numbers, the reagent and media volume should be scaled appropriately.
- We recommend the use of BPS Bioscience TCellM™ and IL-2 for best results.
- This protocol has been optimized for use with T cells isolated from PBMCs with the Human T Cell Isolation Kit (#82288). If using PBMC or T cells isolated by other methods the protocol may require optimization.

Protocol

1. Prepare fresh Complete T Cell Expansion Medium by adding 200 IU/ml to 1,000 IU/ml of Human Interleukin-2 Recombinant (#90184) to TCellM™ (#78753).

2. Add 5 µl of Human T Cell Activation Reagent to 10 ml of complete T Cell Expansion Medium in a 15 ml tube and maintain in a 37°C water bath. This makes T Cell Activation Medium.

3. If using frozen isolated T cells, thaw 1 x 10^7 T cells for 1-2 minutes in a 37°C bath and transfer the cells to a 15 ml tube containing 10 ml of warm Thaw Media 2.

4. Spin down at 300 *x g* for 5 minutes.

5. Discard the supernatant and resuspend cells in 10 ml of T Cell Activation Medium (1 x 10^6 cells/ml).

6. Transfer cells to the appropriate culture vessel and incubate the cells in a humidified 37°C incubator with 5% CO2 for 48-72 hours.

7. Expand cells following the desired protocols.

   Note: T cell activation can be evaluated by measuring CD25 expression by flow cytometry.

Example Results

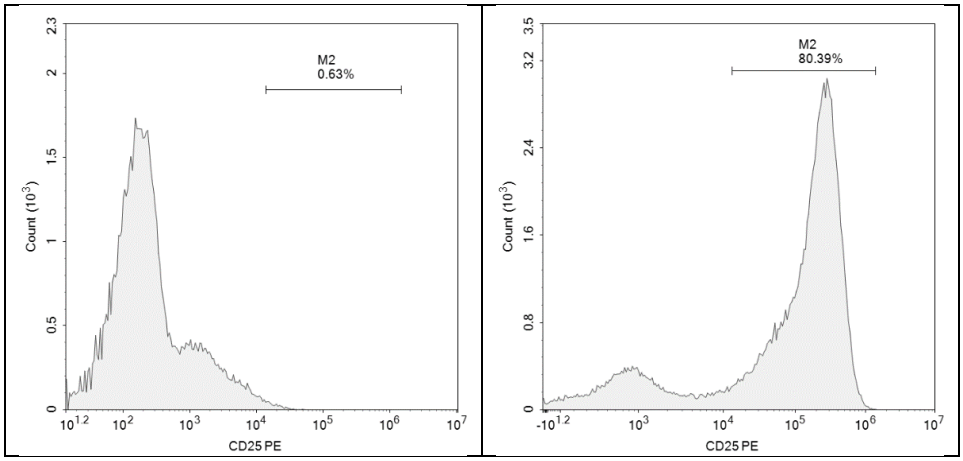

Figure 1: T cell activation marker assessment on isolated Human T Cells activated with Human T Cell Activation Reagent by flow cytometry.
1 x 10^7 isolated human T cells were activated with 5 µl of Human T Cell Activation Reagent and cultured in 10 ml of Complete T Cell Expansion Media (TCellM™ supplemented with 1000 IU/ml of IL-2). Activation of viable $CD3^+$ T cells was measured by assessing CD25 expression on day 0 (left panel) and day 3 (right panel) by flow cytometry with PE anti-human CD25 Antibody (BioLegend #302606). The y axis indicates the % of cells, while the x axis represents PE intensity.

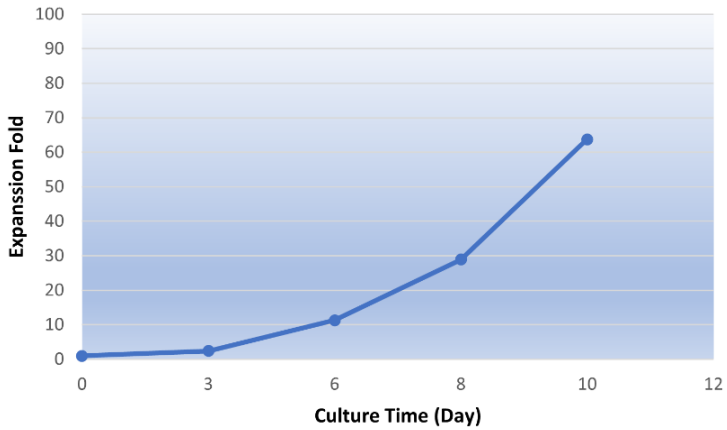

Figure 2: Human T cell expansion following activation with Human T Cell Activation Reagent.
Activated human T cells were expanded over a period of 10 days. On day 0, 10 x 10^6 isolated human T cells were stimulated with 5 µl of Human T Cell Activation Reagent in 10 ml of TCellM™ supplemented with 1000 IU/ml of IL-2 (Complete T Cell Expansion Media). On days 3, 6, 8, and 10, the number of viable cells was counted, and the cell culture was diluted into fresh Complete T Cell Expansion Media only. No additional T Cell Activation Reagent, Human was added during the 10-day culture period. Results are expressed as fold-expansion (in which the number of live cells at day 0 was set to 1). Cell viability remains > %95 throughout the T cell expansion period.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Anti-CD8 Antibody, PE-Labeled	102011	25 µg/100 µg
Anti-CD4 Antibody, PE-Labeled	102010	25 µg/100 µg
$CD4^+$ T Cells, Negatively Selected (Human)	79752	10 million cells
$CD8^+$ T Cells, Negatively Selected (Human)	79753	10 million cells
Anti-BCMA CAR-T Cells	78660	1 vial/5 vials
Anti-CD19 CAR-T Cells	78711	1 vial/5 vials

Version 040524