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Dynabeads® Human T-Expander CD3/CD28

Catalog no. 11141D **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 006)

Product Contents

Product contents	Volume
Dynabeads® Human T-Expander CD3/CD28	10 mL

10 mL of Dynabeads® Human T-Expander CD3/CD28 supplied as a suspension containing 1 × 10^8 beads/ mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% human serum albumin (HSA).

Product Description

Dynabeads® Human T-Expander CD3/CD28 are intended for separation and *in vitro* expansion of human T cells. This product is the research version of Dynabeads® CD3/CD28 CTS™. Human T-Expander CD3/CD28 offer a simple method for isolation and expansion of human T cells. Firstly, $CD3^+$ T cells are separated and concentrated from the apheresis product by magnetic cell separation using Dynabeads® Human T-Expander CD3/CD28. Following separation, the $CD3^+$ T cells are cultured in the presence of the beads. By combining anti-CD3 and anti-CD28 antibodies on Dynabeads®, the beads will provide both the primary and co-stimulatory signals that are required for activation and expansion of T cells. Activated T cells produce IL2, GM-CSF, IFN-g and TNF-α. T cells activated with Dynabeads® Human T-Expander CD3/CD28 can be expanded 100–1000-fold over a 9–14 day culture period.

Downstream Applications

The activated T cells can be analyzed shortly after activation (for transfection/ transduction or to study e.g. T cell receptor signaling, proteomics or gene expression). T cells can be left in culture to differentiate into T helper cell subsets, T cell proliferation, or expansion of polyclonal.

For activation of regulatory T cells or antigen-specific T cells, see "Related Products".

Visit www.lifetechnologies.com and search for the cat. no. (11141D) to get the latest references.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for magnet recommendations. (DynaMag™ CTS™ is recommended for larger volumes where the PBMC and beads are in bags).
- Sample mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Dynabeads® M-450 Epoxy.
- Dulbecco's PBS (DPBS).
- Pooled Human Serum (HS) heat inactivated at 56°C for 1 hour.
- Human Serum Albumin (HSA).
- OpTmizer™ T-Cell Expansion SFM (or equivalent).
- RPMI-1640.
  - L-glutamine.
  - HEPES buffer.
- IL-2 Recombinant Human.
- Appropriate sterile culture vessels.

Media Preparation

- **Buffer 1:** PBS, pH 7.4, with 5% heat-inactivated human serum or 1% HSA.
- **Incomplete Medium:** OpTmizer™ T-Cell Expansion SFM 1X formulation designed to support the culture and expansion of human T cells (equivalent).
- **Complete Medium:** Add 200 IU/mL IL-2 to the incomplete medium. Store at 2°C to 8°C for a maximum of 7 days.
  Note: Autologous serum may be used, but expect donor-to-donor variation. IL-2 concentrations can be increased to enhance cell growth, although in static culture there is no significant increase in expansion for normal T cells when IL-2 levels are increased beyond 200–500 IU. Regulatory T cells may benefit from use of IL–2 at ≥500 IU/mL. For IL-2 products where activity is not available in IU, typically 6–10 ng/mL bioactive IL-2 is sufficient for optimal T cell expansion.

Protocol

Wash Dynabeads®

Wash Dynabeads® before use.

1. Resuspend the Dynabeads® in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads® to a tube.
3. Add the same volume of Buffer 1, or at least 1 mL, and resuspend.
4. Place the tube on a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Dynabeads® in the same volume of Buffer 1, as the initial volume of Dynabeads® (step 2).

Starting Material

The preferred starting material is cryopreserved human PBMC obtained from leukapheresis product or Ficoll® separated whole blood. The starting material can also be enriched for specific T cell subsets, such as $CD4^+$ T cells or $CD25^+$ T cells.

Alternatively, PBMC from freshly obtained leukapheresis products or Ficoll® separated whole blood may be activated and expanded without cryopreservation. For maximum activation and expansion of T cells in PBMC magnetically capture $CD3^+$ T cells prior to culture initiation (see "Magnetic Separation and Expansion of $CD3^+$ T cells"). Magnetic concentration is not required if T cells or T cell subsets have been enriched prior to activation and expansion (see "Culture Initiation Without Magnetic Concentration of $CD3^+$ T Cells").

Magnetic Separation and Expansion of $CD3^+$ T cells

Note: Monocytes can rapidly phagocytose beads at 37°C, thereby reducing the absolute number of beads capable of contacting T cells, in turn reducing the level of T cell activation and expansion.

1. Determine the percentage of $CD3^+$ T cells in the sample by flow cytometry or other suitable methods.
2. For PBMC, resuspend cells in a tube at roughly 2–5 × 10^7 $CD3^+$ cells/mL in Buffer 1, but not exceeding a maximum of 2 × 10^8 total nucleated cells/mL. For previously enriched/purified T cells (e.g. $CD4^+$), resuspend the cells at 5–10 × 10^7 cells/mL.
3. Add washed Dynabeads® Human T-Expander CD3/CD28 at a 3:1 bead: $CD3^+$ T cell ratio.
   Note: Bead: $CD3^+$ T cell ratio can be dropped as low as 1:1 when the starting T cell pool is more prone to activation induced cell death or where short-term activation is desired.
4. Rotate the sample at 1 rpm for 30 min at between 4°C to 25°C. Optimize the mixing temperature between 4°C to 25°C for each application.

5. Increase the volume in the tube to sufficient levels for magnetic selection by adding Incomplete Medium or Buffer 1 before placing the tube in a magnet for 1–2 min.
6. Remove supernatant and stain with anti-CD3 antibody for flow cytometric analysis to calculate depletion efficiency.
7. Gently resuspend bead-cell complexes to an estimated $0.5 \times 10^6 – 1 \times 10^6$ $CD3^+$ T cells/mL in Complete Medium.
8. Plate cells at $2.5–5 \times 10^6$ $CD3^+$ cells in a total volume of 5 mL/well ($0.5–1.0 \times 10^6$ cells/mL) of a 6 well culture plate in a humidified incubator at 37°C/ 5% CO_2 for 3 days.
   Note: Smaller or larger culture vessels/wells can be used; adjust volumes accordingly. It is important that static cultures do not exceed ~1.2 cm in volume depth, otherwise expansion potential and viability may be compromised due to reduced gas exchange.

Counting and Splitting of Cultures

Evaluate cell concentration daily, beginning on day 3 of culture. It may be useful to conduct regular phenotypic analyses by flow cytometry (e.g. days 3, 5, 8, and end of culture) to track T cell purity, phenotype and activation state. Similarly, supernatants may be collected to measure cytokine secretion patterns to further characterize T cell activity.

1. Gently mix contents of wells to dissociate beads and cells. Mixing should be sufficient to disrupt all visible bead:cell complexes.
2. Remove 50 µL sample and mix in 50 µL trypan blue (do not remove beads before counting).
3. Count cells manually using a hemocytometer.
4. When $CD3^+$ T cell density is $>1 \times 10^6$ cells/mL, dilute cells to approximately 0.5×10^6 $CD3^+$ T cells/mL in culture medium.
5. At the end of culture (day 9–14) count cells and remove beads with a magnet.

Culture Initiation Without Magnetic Concentration of $CD3^+$ T Cells

1. Add 5×10^6 enriched or purified T cells to 1.5×10^7 washed Dynabeads® Human T-Expander CD3/CD28 in a final volume of 10 mL culture media, placing 5 mL/ well in a 6-well tissue culture plate to give a final concentration of 0.5×10^6 $CD3^+$T cells/mL. Mix gently.
2. Culture cells in a humidified incubator at 37°C/5% CO_2 for 3 days.
3. Cell concentration should be evaluated daily beginning on day 3 of culture.
4. For counting of cells and splitting of culture; see section “Counting and Splitting of Cultures”.

Procedures Incorporating Gene Transduction

Typically, for all culture conditions described earlier, T cells from normal donor tissues begin cycling and start to divide between day 2 and 3 of culture. Days 2, 3, and/or 4 of culture are recommended as optimal days for transduction using moloney-based vectors, whereas day 1, 2, and/or 3 are recommended for lentivirus based vectors. Magnetic removal of beads prior to transduction will diminish overall cell expansion, but should not affect viability. Leaving beads in during the retroviral transduction process should be acceptable for most transduction applications.
Note: T cells obtained from patients with various diseases and/or undergoing various treatments may be slower to enter cell cycle and cell division may not commence until 1, 2 or even 3 days later than typically observed for tissues from healthy donors. Thus it is important to monitor T cell activation markers, such as CD25, as well as cell division to determine optimal splitting schedules and timing for gene modification.

Description of Materials

Dynabeads® Human T-Expander CD3/CD28 are uniform 4.5 µm, superparamagnetic polystyrene beads coated with a mixture of monoclonal antibodies against the CD3 and CD28 cell surface molecules of human T cells.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™–15	12301D
DynaMag™–50	12302D
Dynabeads® M-450 Epoxy	14011
HulaMixer® Sample Mixer	15920D
Dulbecco's Phosphate Buffered Saline	14190-094
Advanced RPMI Medium 1640	12633-012
OpTmizer™ T-Cell Expansion SFM	0080022SA
RPMI-1640	12633-012
L-glutamine	25030-0832
HEPES buffer	15630-080
IL-2 Recombinant Human	PHC0023

REF on labels is the symbol for catalog number.

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