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invitrogen bead separations

Cat. no. 111.31D
111.32D

Rev. no. 004

Dynabeads® CD3/CD28 T Cell Expander

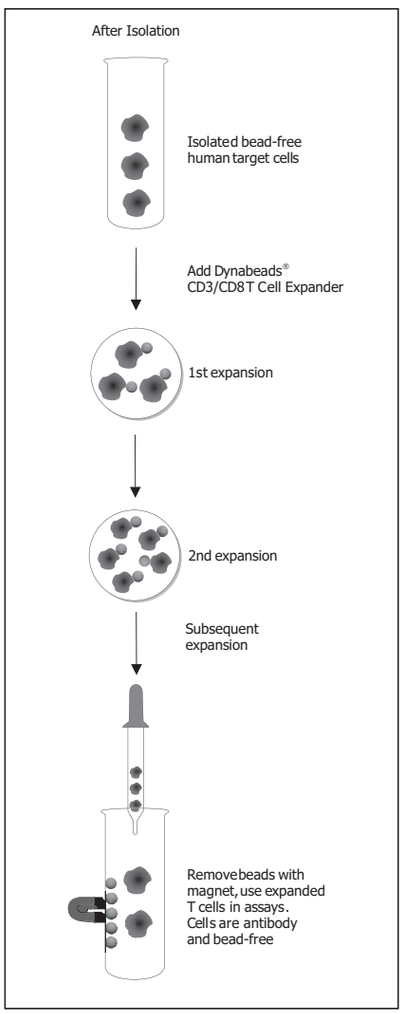
For research use only

Index

1 PRODUCT DESCRIPTION

1.1 Intended Use

This product is intended for activation and expansion of human T cells and T cell clones. T cell clones ($CD28^+$) retain specificity and functional properties after repeated re-stimulation with Dynabeads® CD3/CD28 T Cell Expander.



1.2 Principle of Activation and Expansion

Dynabeads CD3/CD28 T Cell Expander offers a simple method for activation and expansion of T cells that does not require antigen-presenting cells or antigen. Just add the Dynabeads for activation or Dynabeads plus recombinant IL-2 (rIL-2) for expansion of T cells. Cell cultures showing signs of exhaustion can be re-stimulated by adding fresh beads and rIL-2.

Dynabeads CD3/CD28 T Cell Expander offer the first artificial antigen-presenting cells to provide simultaneous signals to TCR/CD3 and CD28 for full activation and expansion of human T cells.

1.3 Description of Materials

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

The CD3 antibody coated on the CD3/CD28 T Cell Expander is specific for the epsilon chain of human CD3, a subunit of the TCR complex. The CD28 antibody is specific for the human CD28 co-stimulatory molecule, which is the receptor for CD80 (B7-1) and CD86 (B7-2). Both antibodies are coupled to the same Dynabead, mimicking *in vivo* stimulation by antigen presenting cells.

The plasma used for human serum albumin (HSA) in this product is tested and found free of Human Immunodeficiency Virus (HIV-1 and HIV-2), Hepatitis-B Virus (HBV) and Hepatitis-C Virus (HCV).

Materials Supplied

2 ml or 5 x 2 ml Dynabeads CD3/CD28 T Cell Expander:

4 x 10^7 beads/ml in phosphate buffered saline (PBS), pH 7.4, w/0.1% human serum albumin (HSA).

Additional Materials Required

Materials that are not included, but are needed to perform the entire protocol:

- Magnet (Dynal MPC™): See www.invitrogen.com/magnets-selection for magnet recommendations.
- Buffer 1: PBS w/ 0.1% BSA, pH 7.4.
- Culture Medium: OpTmizer™ T-Cell Expansion SFM (Gibco)serum free 1x formulation designed to support the culture and expansion of human T cells (or equivalent).
- rIL-2.

2. PROTOCOLS

2.1 Technical Advice

- Do not use excess volume of Dynabeads as extra beads per well will inhibit cell growth.

2.2 Dynabeads Washing Procedure

Dynabeads should be washed before use.

1. Resuspend the Dynabeads in the vial.
2. Transfer the desired volume of Dynabeads to a tube.
3. Add the same volume of Buffer 1, or at least 1 ml, and mix.
4. Place the tube in a magnet for 1 minute 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).

2.3 Isolation of Human T Cells

For isolation of human T cells it is recommended to use the Dynal T Cell Negative Isolation Kit (Cat. no. 113.11D), Dynal CD4 Negative Isolation Kit (Cat. no. 113.17D), Dynal CD8 Negative Isolation Kit (Cat. no. 113.19D), Dynal CD4 Positive Isolation Kit (Cat. no. 113.31D) or Dynal CD8 Positive Isolation Kit (Cat. no. 113.33D).

2.4 Short Term Stimulation of Human T Cells (1-3 days)

For short term culture of T cells for 1 - 3 days Invitrogen Dynal recommends using 1 bead per T cell. Short-term culture can be performed without adding exogenous rIL-2.

MNC or T cells can be used for short term stimulation with Dynabeads CD3/CD28 T Cell Expander.

Day 0:

1. Add MNC or T cells to the culture medium at 1 x 10^6 cells/ml.
2. Add an equal number of Dynabeads CD3/CD28 T Cell Expander to the cells, 25 µl beads to 1 x 10^6 T cells.
3. Leave for 1-3 days in an incubator.

2.5 Polyclonal Expansion of T Cells

For expansion of T cells for >3 days Dynal recommends using 3 beads per T cell.

MNC or T cells can be used for expansion with Dynabeads CD3/CD28 T Cell Expander.

Day 0:

1. Add MNC or T cells to the culture medium at 1 x 10^6 cells/ml.
2. Add 75 µl Dynabeads CD3/CD28 T Cell Expander to 1 x 10^6 cells.
3. Leave in an incubator.

Day 2-3:

Add 10-100 U rIL-2/ml.

Day 4-5:

1. Resuspend the cells and beads with a pipette and count the cells in a microscope.
2. Dilute the cells to 0.5 x 10^6 cells/ml in culture medium containing 10-100 U rIL-2/ml.

Day 7-14:

Split the culture when needed. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures. Count the cells at least twice weekly after thorough resuspension. When the cell density exceeds 2 x 10^6 cells/ml or when the medium becomes yellow, split cultures to a density of 0.5 x 10^6 cells/ml in culture medium containing 10-100 U rIL-2/ml.

Day 14: Cells can usually be expanded for up to 2 weeks before restimulation is needed.

Re-stimulation:

1. Resuspend cells and beads before removing beads with a magnet.
2. Count the cells and dilute cells to 0.5 x 10^6 cells/ml in culture medium containing 10-100 U rIL-2/ml.
3. Re-stimulate by adding new Dynabeads CD3/CD28 T Cell Expander, 3 beads per T cell.
4. Culture cells for 2 - 3 days, count and dilute cells as described above.
5. Re-stimulation with Dynabeads CD3/CD28 T Cell Expander can be performed several times, as long as the T cells express CD28.

2.6 Expansion of T Cell Clones

Day 0:

1. Transfer $CD4^+CD28^+$ or $CD8^+CD28^+$ T cell clones from a full Terasaki well to a 96 well plate.
2. Add 0.125 µl Dynabeads CD3/CD28 T Cell Expander and 10-100 U rIL-2/ml per well. (1 bead per 5 - 10 T cells, higher number of beads will reduce expansion of T cell clones).
3. Leave in an incubator.

Day 5-9: Grow the clones until the well is half-full (~500,000 cells). If wells turn yellow during culture, add 50-100 µl medium with 10-100 U rIL-2/ml. If higher cell numbers are needed, continue with restimulation as described below.

Re-stimulation:

Day 0:

1. Resuspend cells and beads before removing beads with a magnet.
2. Count the cells and dilute cells to 0.25 - 0.5 x 10^6/ml in culture medium containing 10-100 U rIL-2/ml. Transfer the cloned cells from a 96 well plate to a 24 well plate. If estimated cell number is below 0.25 x 10^6 cells transfer to a 48 well plate.
3. Re-stimulate by adding 1.25 µl Dynabeads CD3/CD28 T Cell Expander per 24 well (1 bead per 5-10 T cells) or 0.63 µl Dynabeads to a 48 well plate.
4. Leave in an incubator.

Day 3-14:

Split the culture when needed. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation rate is typically observed in exhausted cell cultures. Count the cells at least twice weekly after thorough resuspension. When the cell density exceeds 2 x 10^6 cells/ml or when the medium becomes yellow, split cultures to a density of 0.5 x 10^6 cells/ml in culture medium containing 10-100 U rIL-2/ml.

Further re-stimulation can be performed with fresh Dynabeads CD3/CD28 T Cell Expander as described above.

3. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

Storage & Precautions

This kit is stable until the expiry date stated on the label, when stored unopened at 2-8°C

Store opened vials at 2-8°C and avoid bacterial contamination

Keep Dynabeads in liquid suspension during storage and all handling steps, since drying will result in reduced performance. Resuspend well before use.

Technical Service

Please contact Invitrogen Dynal for further technical information at www.invitrogen.com.

Certificate of Analysis (CoA) is available upon request.

Warnings and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

Patents and Trademarks

Intellectual Property Disclaimer

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The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

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