DYNAL® BIOTECH

Prod. No. 113.11

Rev. No. 003

Dynal® T Cell Negative Isolation Kit

For research use only.

This kit depletes activated T cells.

1. PRODUCT DESCRIPTION

1.1 Intended Use

Isolate untouched human T cells by depleting non-T cells (B cells, NK cells, monocytes, platelets, dendritic cells, granulocytes, erythrocytes) and activated T cells from peripheral blood. Isolated T cells are bead- and antibody-free and are suitable for any downstream application.

This kit will deplete activated T cells (HLA class II positive cells). If you do not want to deplete activated T cells, please use the Dynal T Cell Negative Isolation Kit Ver II (Prod. No. 113.37).

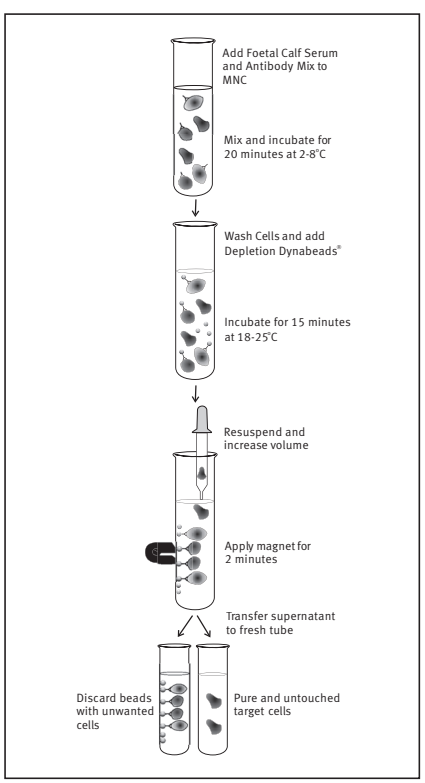

Fig. 1 Simple method for isolating untouched human T cells.

1.2 Principle of Isolation

Add a mixture of monoclonal antibodies against the non-T cells to the starting sample. Add Dynabeads to bind to the non-T cells during a short incubation. Separate the bead-bound cells by a magnet. Discard the bead-bound cells and use the remaining, untouched human T cells for any application.

1.3 Description of Materials

Dynabeads are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a monoclonal human anti-mouse IgG antibody. The antibody coated onto Dynabeads recognises all mouse IgG subclasses and is Fc-specific. The antibody on the Dynabeads is a human IgG4 anti-mouse IgG. The source of the human monoclonal antibody is free of Human Immunodeficiency Virus (HIV), Hepatitis-B Virus (HBV) and Hepatitis-C Virus (HCV).

Materials Supplied

Dynal T Cell Negative Isolation Kit contains Depletion Dynabeads and Antibody Mix.

- **5 ml Depletion Dynabeads.**
  Supplied in phosphate buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.
- **1 ml Antibody Mix.**
  The Antibody Mix contains mouse IgG antibodies for CD14, CD16 (specific for CD16a and CD16b), HLA Class II DR/DP, CD56 and CD235a (Glycophorin A).

  Supplied in PBS and 0.02% sodium azide (NaN_3).
- **The kit will process up to 5 x 10^8 cells.**

Additional Materials Required

- Magnet: (Dynal MPC) - MPC-L for 1-5 ml samples, MPC-15 for 1-15 ml samples and MPC-50 for 15-50 ml samples.
- Mixer allowing both tilting and rotation.
- Heat inactivated Foetal Calf Serum (FCS).
- Buffer 1: PBS (without Ca^{2+} and Mg^{2+}) w/0.1% BSA and 2 mM EDTA, pH 7.4.
- Buffer 2: PBS (without Ca^{2+} and Mg^{2+}), pH 7.4.
- Lymphoprep™ for MNC preparation (Axis Shield PoC, Norway, www.axis-shield-poc.com).

Keep the buffers cold!

Important Notes:

BSA can be replaced by human serum albumin (HSA) or FCS.

EDTA can be replaced by sodium citrate.

PBS containing Ca^{2+} or Mg^{2+} is not recommended.

2. PROTOCOLS

2.1 Dynabeads Washing Procedure

Dynabeads should be washed before use.

1. Resuspend the Dynabeads in the vial to a homogenous suspension.
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 2 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.

2.2 Preparation of MNC from Buffy Coat to Obtain Low Platelet Numbers

1. Dilute 10 - 18 ml buffy coat with Buffer 2 to a total volume of 35 ml at 18-25°C (RT).
2. Add the diluted buffy coat on top of 15 ml of Lymphoprep.
3. Centrifuge at 160 x g for 20 min at RT. Allow to decelerate without brakes.
4. Remove 20 ml of supernatant to eliminate platelets.
5. Centrifuge at 350 x g for 20 min at RT. Allow to decelerate without brakes.
6. Recover MNC from the plasma/Lymphoprep interface and transfer the cells to a 50 ml tube.
7. Wash MNC once with Buffer 1 by centrifugation at 400 x g for 8 min at 2-8°C.
8. Wash MNC twice with Buffer 1 by centrifugation at 225 x g for 8 min at 2-8°C and resuspend the MNC at 1 x 10^8 MNC per ml in Buffer 1.

For other recommended sample preparation procedures, visit http://www.dynalbiotech.com/samplepreparation

2.3 Critical Steps for Cell Isolation

- Use a mixer that provides tilting and rotation of the tubes to ensure Dynabeads do not settle at the bottom of the tube.
- Never use less than 100 µl Dynabeads per 1 x 10^7 MNC sample.
- It is critical to follow the magnet recommendations to ensure a successful isolation.

2.4 Isolation of Human T Cells from MNC

This protocol is based on 1 x 10^7 MNC. It is scalable from 1 x 10^7-5 x 10^8 cells, (see table 1).

1. Transfer 100 µl (1 x 10^7) MNC in Buffer 1 to a tube.
2. Add 20 µl heat inactivated FCS.
3. Add 20 µl of Antibody Mix.
4. Mix well and incubate for 20 min at 2-8°C.
5. Wash the cells by adding 2 ml Buffer 1. Mix well by tilting the tube several times and centrifuge at 300 x g for 8 min at 2-8°C. Discard the supernatant.
6. Resuspend the cells in 900 µl Buffer 1.
7. Add 100 µl pre-washed Depletion Dynabeads
8. Incubate for 15 min at 18-25°C with gentle tilting and rotation.
9. Resuspend the bead-bound cells by **gently** pipetting 5 times using a pipette with a narrow tip opening, (e.g. a 1000 µl pipette tip or a 5 ml serological pipette).
10. Add 1 ml Buffer 1.
11. Place the tube in the magnet for 2 min.
12. Transfer the supernatant to a new tube.
13. Repeat steps 10-12.

The supernatant contains the negatively isolated human T cells.

Table 1. Volumes for human T cell isolation (1 x 10^7 to 5 x 10^8 MNC)

	Working volume per 1 x 10^7 MNC
Cell volume (step 1)	100 µl
FCS (step 2)	20 µl
Antibody Mix (step 3)	20 µl
Washing (step 5)	2 ml (Max. 40 ml when scaled up)
Resuspension (step 6)	900 µl Max. 5 ml when scaled up)
Depletion Dynabeads (step 7)	100 µl
Increase volume (step 10)	1 ml (Max. 35 ml when scaled up)
Dynal MPC recommended	MPC-L/MPC-15/MPC-50

2.5 Downstream Applications

Isolated T cells can be used in many applications such as cell culture, flow cytometry, functional assays and molecular studies.

3. GENERAL INFORMATION

Storage/Stability

This kit is stable until the expiry date stated on the label when stored unopened at 2-8ºC.

Store opened vials at 2-8ºC and avoid bacterial contamination.

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

Technical Support

Please contact Dynal Biotech for further technical information (see contact details).

Warning And Limitations

This kit is for research use only.

Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide as a preservative, which is cytotoxic. **Avoid pipetting by mouth!** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build up.

Certificate of Analysis (CoA) is available upon request.

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

Patents and Trademarks

Several international patents and patent applications cover the production and use of the Dynabeads products.

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4. REFERENCES

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