Miltenyi Biotec

T Cell Activation/ Expansion Kit
mouse

Order no. 130-093-627

Contents

1. Description

This product is for research use only.

Components	**2 mL Anti-Biotin MACSiBead Particles, cell culture grade,** corresponding to 4×10^8 MACSiBead Particles; MACSiBead Particles conjugated to monoclonal anti-biotin antibodies. **0.4 mL CD3ε-Biotin, mouse** (100 µg/mL) **0.4 mL CD28-Biotin, mouse** (100 µg/mL)
Product format	All components are supplied in azide-free buffer containing stabilizer. Low endotoxin.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the T Cell Activation/Expansion Kit

The T Cell Activation/Expansion Kit is designed to activate and expand mouse T cells. The kit consists of Anti-Biotin MACSiBead Particles and biotinylated antibodies against mouse CD3ε and CD28. Anti-Biotin MACSiBead Particles loaded with biotinylated antibodies are used to mimic antigen-presenting cells and activate resting T cells in single-cell suspensions from spleen or lymph nodes as well as purified T cells. T cell expansion is achieved by culturing and reactivation at day 6–8 of culture.

1.2 Background information

The Anti-Biotin MACSiBead Particles are in a first step loaded with biotinylated antibodies. Best activation and expansion is achieved by using equal amounts of the provided biotinylated antibodies against CD3ε and CD28.

▲ **Note:** Other combinations of biotinylated antibodies may be experimentally tested for their suitability, if required.

Loaded Anti-Biotin MACSiBead Particles are subsequently used for the activation and expansion of T cells. Best stimulation of T cells is accomplished by using 1–2 loaded Anti-Biotin MACSiBead Particles per cell (bead-to-cell ratio 1:1 or 2:1) supplemented with recombinant interleukin 2 (IL-2).

▲ The efficiency of the T cell activation depends on the T cell differentiation status, which will often be heterogeneous. For special applications, it is therefore recommended to experimentally determine the best bead-to-cell ratio and concentrations of IL-2 in the cell culture medium.

▲ Over-activation of T cells carries a risk of activation-induced cell death.

T cells, activated by using Anti-Biotin MACSiBead Particles, can be used for any downstream processing such as cytokine analysis or immunoprecipitation. Also, activated T cells can be transfected with high efficiency.

Anti-Biotin MACSiBead Particles show no autofluorescence and normally do not need to be removed prior to flow cytometric analysis. However, if desired, removal of Anti-Biotin MACSiBead Particles is easily achieved by using the MACSiMAG™ Separator (refer to 2.5).

1.3 Applications

- Activation and expansion of resting mouse T cells (e.g. from splenocytes).
- Activation and expansion of T cells positively selected with MACS® MicroBeads, e.g., CD4 (L3T4) MicroBeads, mouse (# 130-117-043) or CD8 (Ly-2) MicroBeads, mouse (# 130-117-044), or untouched T cells isolated using MACS Pan T Cell Isolation Kit II, mouse (# 130-095-130), $CD4^+$ T Cell Isolation Kit, mouse (# 130-104-454), or $CD8^+$ T Cell Isolation Kit, mouse (# 130-104-075).

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, supplemented with 2 mM EDTA. Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Medium: RPMI 1640 or TexMACS™ Medium, research grade (# 130-097-196) supplemented with 10% FBS and Mouse IL-2 IS, premium grade (# 130-120-332; 30–100 U/mL).

  ▲ **Note:** 2-Mercaptoethanol (0.01 mM) can be added to preserve cell viability in case of rapid cell growth.

- Humidified incubator.
- MACSmix™ Tube Rotator (# 130-090-753) for loading of MACSiBead Particles.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-002-627.04

- (Optional) MACSiMAG™ Separator for removal of Anti-Biotin MACSiBead Particles after T cell expansion prior to downstream experiments.

  ▲ **Note:** Do not remove MACSiBead Particles by using MACS Columns and MiniMACS™, MidiMACS™, SuperMACS™, autoMACS®, or autoMACS Pro Separators.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, for example, CD4-FITC, CD8a-PE, or CD25-Vio® Bright FITC. For more information about other fluorochrome conjugates refer to www.miltenyibiotec.com/antibodies.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for the flow cytometric exclusion of dead cells.

- (Optional) Recombinant interleukin-2 (IL-2), e.g., Mouse IL-2, research grade (# 130-094-054).

2. Protocol

▲ All steps in the protocol have to be performed under sterile conditions.

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid organs using a standard preparation method. General protocols for sample preparation are available at www.miltenyibiotec.com/protocols.

2.2 Loading of Anti-Biotin MACSiBead™ Particles

▲ Resuspend Anti-Biotin MACSiBead Particles thoroughly by vortexing **before use**, to obtain a homogenous suspension.

▲ Anti-Biotin MACSiBead Particles are supplied without preservative. Remove aliquots under aseptic conditions.

▲ It is recommended to load Anti-Biotin MACSiBead Particles in batches of 1×10^8 Anti-Biotin MACSiBead Particles. Loaded Anti-Biotin MACSiBead Particles are stable for up to 4 months when stored at 2–8 °C.

1. Pipette 100 µL of CD3ε-Biotin and 100 µL CD28-Biotin into a sealable 2 mL tube and mix well.

   ▲ **Note:** This antibody combination, with a final antibody concentration of 10 µg antibody per 1 mL loaded Anti-Biotin MACSiBead Particles, is optimized for achieving maximum T cell activation.

2. Add 300 µL of buffer and mix well.

3. **Resuspend** Anti-Biotin MACSiBead Particles **thoroughly** by vortexing.

4. Remove 500 µL Anti-Biotin MACSiBead Particles (1×10^8 Anti-Biotin MACSiBead Particles) and add to antibody mix.

   ▲ **Note:** Anti-Biotin MACSiBead Particles can be loaded in a flexible manner with biotinylated antibodies or ligands other than those supplied. If desired, add other biotinylated antibodies or ligands at appropriate concentrations and adjust with buffer to a total volume of 1 mL accordingly.

5. Incubate for 2 hours at 2–8 °C under constant, gentle rotation by using the MACSmix Tube Rotator (# 130-090-753) at approximately 4 rpm (slowest permanent run program).

6. The **loaded Anti-Biotin MACSiBead Particles** (1×10^8 Anti-Biotin MACSiBead Particles/mL) are now ready to use. **Do not remove the loaded Anti-Biotin MACSiBead Particles from the antibody mix.** Store at 2–8 °C for up to 4 months.

2.3 T cell activation and expansion protocol

▲ Volumes for activation and expansion given below are for 2×10^6 lymphocytes or isolated cells and 2×10^6 or 4×10^6 Anti-Biotin MACSiBead Particles (bead-to-cell ratio 1:1 or 2:1), depending on the experimentally determined best bead-to-cell ratio for the respective cells and mouse models. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 4×10^6 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend **loaded Anti-Biotin MACSiBead Particles** thoroughly and transfer 20 µL or 40 µL (2×10^6 or 4×10^6 loaded Anti-Biotin MACSiBead Particles) per 2×10^6 cells to a suitable tube.

   ▲ **Note:** If unloaded MACSiBead Particles shall be used for negative control experiments, replace loaded Anti-Biotin MACSiBead Particles by adding 20 µL or 40 µL (2×10^6 or 4×10^6 beads) of unloaded Anti-Biotin MACSiBead Particles per 2×10^6 cells.

2. Add 1 mL of culture medium to the **loaded Anti-Biotin MACSiBead Particles** and centrifuge at 300×g for 5 minutes.

3. Aspirate supernatant and resuspend **loaded Anti-Biotin MACSiBead Particles** in 1 mL of fresh culture medium (e.g. RPMI 1640 or TexMACS™ Medium) supplemented with 10% FBS and 30–100 units IL-2/mL.

   ▲ **Note:** Addition of low amounts of IL-2 results in a higher expansion rate of $CD4^+$ T cells compared to $CD8^+$ T cells, whereas addition of higher amounts of IL-2 results in a higher expansion rate of $CD8^+$ T cells.

4. Resuspend cells at a density of 2×10^6 cells per mL of culture medium in RPMI 1640 or TexMACS Medium supplemented with 10% FBS and IL-2.

   ▲ **Note:** Defined media, e.g., TexMACS Medium, might be advantageous for T cell expansion.

5. Add the cell suspension and the prepared Anti-Biotin MACSiBead Particles from step 3 to a suitable cell culture vessel at a density of 1×10^6 cells per mL per cm^2 (e.g. 2×10^6 cells in 2 mL/well of a 24-well plate).

   ▲ **Note:** Refer to 4. Appendix: Flask and dish sizes for *in vitro* T cell expansion.

6. Incubate at 37 °C and 5–10% CO_2 for up to 2 days.

   ▲ **Note:** Inspect cultures daily, and add fresh medium if required.

7. At day 2, gently pipette culture up and down to break up all cell clumps.

8. Split the cell culture every two days 1:4 or 1:2, depending on the proliferation of cells, and add fresh culture medium supplemented with 10% FBS and IL-2 (1×10^6 cells per mL per cm^2).

   ▲ **Note:** Daily inspect the culture. Depending on the expansion rate, it might be necessary to split culture more frequently than every 2 days.

9. After 6–8 days of activation, T cells are in a resting state and further expansion of T cells requires a restimulation.

2.4 Immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for 10^6 total cells. When working with fewer than 10^6 cells and up to 10^7 cells, use the same volumes as indicated.

▲ MACSiBead Particles show no autofluorescence and do not need to be removed prior to flow cytometric analysis.

▲ After stimulation for up to 24 h, scatter properties of cells may be altered due to strong interaction between cells and MACSiBead Particles.

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

140-002-627.04

▲ Upon stimulation, expression of CD3 might be transiently down-regulated. Thus, the staining of CD3 on the cell surface of activated cells might be affected.

1. Resuspend cells to break up cell clumps.

   ▲ **Note:** During short-term stimulation of cells (up to 24 hours), Anti-Biotin MACSiBead Particles may be bound strongly to cells. Care should be taken to thoroughly resuspend cells before analysis.

2. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 10 µL of each staining antibody, e.g., CD4-FITC, to 10^6 cells resuspended with buffer to a total volume of 110 µL.
4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Higher temperatures and/or longer incubation times lead to non-specific cell labeling. Working on ice requires increased incubation time.

5. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

2.5 Removal of Anti-Biotin MACSiBead™ Particles

▲ Removal of MACSiBead Particles may be required before magnetic separation of cells with MACS® MicroBeads or before restimulation with different agents or antigens.

1. Harvest cells and transfer to a 5 mL, 15 mL, or 50 mL tube and wash once with buffer.
2. Resuspend cells in buffer at a density of up to 2×10^7 cells per 1 mL and vortex thoroughly.
3. Place the tube in the magnetic field of the MACSiMAG™ Separator.

   ▲ **Note:** Use tube rack to insert 5 mL tube into the magnetic field of the separator. For details refer to the MACSiMAG Separator data sheet.

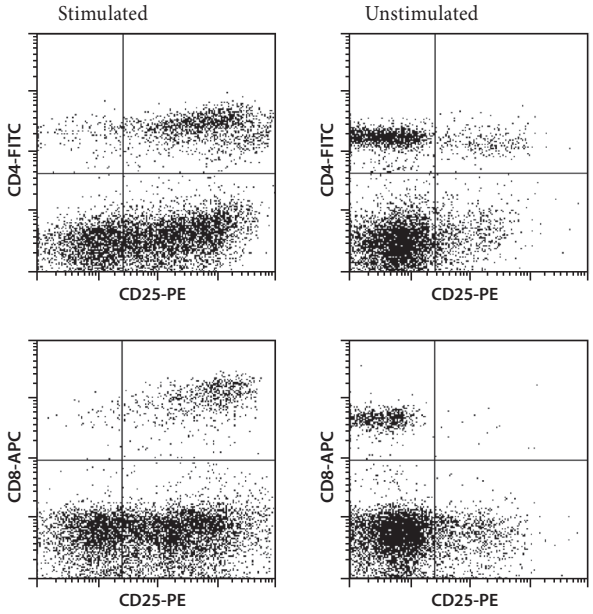
4. Allow the MACSiBead Particles to adhere to the wall of the tube:

5 mL tubes:	2 minutes
15 mL or 50 mL tubes:	4 minutes

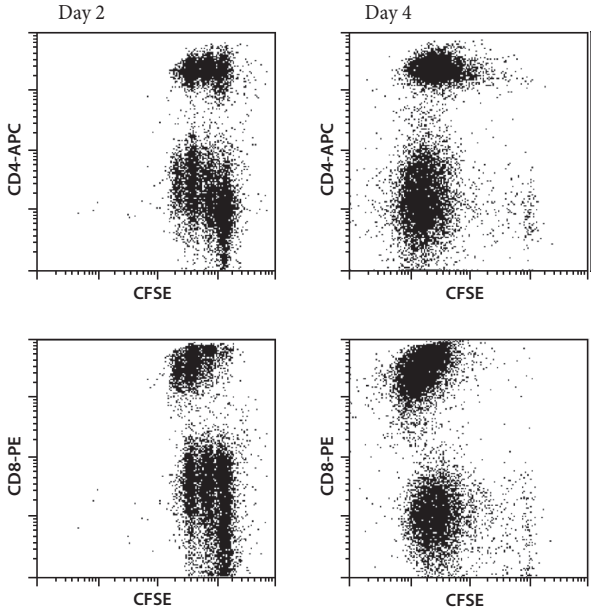
5. Retaining the tube in the magnet, carefully remove the supernatant containing the MACSiBead-depleted cells and place in a new tube.
6. Remove the tube from the separator and add buffer to the same volume as before.
7. Vortex sample, replace tube in the MACSiMAG Separator, and repeat steps 4–5.
8. Collected cells can now be further processed as required.

3. Example of T cell activation and expansion using the T Cell Activation/Expansion Kit

A) Anti-Biotin MACSiBead Particles were loaded with CD3ε- and CD28-Biotin. Mouse spleen cells were activated using 1 loaded Anti-Biotin MACSiBead Particle per 1 lymphocyte, for 20 hours (in RPMI and 10% FBS; without IL-2). The negative control experiment was performed without adding MACSiBead Particles. Cells were fluorescently stained using CD4-FITC, CD8-APC, and CD25-PE. Dead cells and debris were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



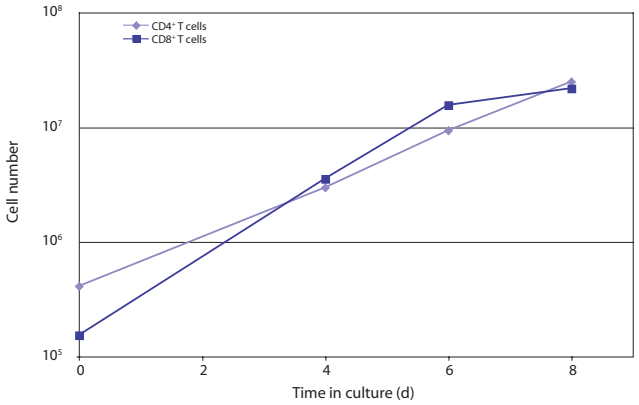
B) Anti-Biotin MACSiBead Particles were loaded with CD3ε- and CD28-Biotin. CFSE-labeled mouse spleen cells were activated and expanded using 1 loaded Anti-Biotin MACSiBead Particle per 1 lymphocyte. The cells were cultured (RPMI supplemented with 10% FBS and 40 U IL-2/mL) at an initial density of 10^6 lymphocytes per mL and cm^2. Cells were fluorescently stained on day 2 and day 4 using CD4-APC and CD8-PE. Dead cells and debris were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

140-002-627.04

C) Anti-Biotin MACSiBead™ Particles were loaded with CD3ε- and CD28-Biotin. Mouse spleen cells were expanded using 1 loaded Anti-Biotin MACSiBead Particle per 1 lymphocyte. The cells were cultured (RPMI supplemented with 10% FBS and 40 U IL-2/mL) at an initial density of 10^6 lymphocytes per mL and cm^2. On days 4, 6, and 8 cells were fluorescently stained for CD4 and CD8 expression and the absolute number of $CD4^+$ and $CD8^+$ T cells was calculated.



4. Appendix: Flask and dish sizes for *in vitro* T cell expansion

For T cell expansion the cells should be resuspended in culture medium at 1×10^6 cells/mL. The cells should be plated at a density of 1×10^6 cells/cm^2. Both the dilution and the cell density are important to assure optimal stimulation and cell growth.

The following table lists culture plate, dish and flask sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to add.

Total cell number	Medium volume to add	Culture plate	Well diameter
0.25×10^6	0.25 mL	96 well	0.64 cm
1.00×10^6	1.00 mL	48 well	1.13 cm
2.00×10^6	2.00 mL	24 well	1.60 cm
4.00×10^6	4.00 mL	12 well	2.26 cm
10.00×10^6	10.00 mL	6 well	3.50 cm
Total cell number	**Medium volume to add**	**Culture dish**	**Dish diameter**
9×10^6	9.0 mL	small	3.5 cm
20×10^6	20.0 mL	medium	6 cm
50×10^6	50.0 mL	large	10 cm
100×10^6	100.0 mL	extra large	15 cm
Total cell number	**Medium volume to add**	**Culture flask**	**Growth area**
24×10^6	24 mL	50 mL	25 cm^2
80×10^6	80 mL	250 mL	75 cm^2
160×10^6	160 mL	720 mL	162 cm^2
240×10^6	240 mL	900 mL	225 cm^2

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, MACS, MACSiBead, MACSiMAG, MACSmix, MidiMACS, the Miltenyi Biotec logo, MiniMACS, SuperMACS, TexMACS, and Vio are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2020 Miltenyi Biotec and/or its affiliates. All rights reserved.