Ova Antigen Delivery Reagent

Order no. 130-094-663

Contents

1. **Description**
   - 1.1 **Background information**
   - 1.2 **Applications**
   - 1.3 **Reagent requirements**
2. **Protocol**
   - 2.1 **Sample preparation**
   - 2.2 ***In vitro* targeting using Ova Antigen Delivery Reagent**
3. **Examples of using the Ova Antigen Delivery Reagent**
4. **References**

1. Description

Components	200 µL Ova Antigen Delivery Reagent: monoclonal Anti-Biotin antibodies (isotype: mouse IgG1) conjugated to ovalbumin (Ova) and fluorescein isothiocyanate (FITC).
Capacity	20 tests or up to 2×10^7 total cells.
Product format	Antibodies are supplied in buffer containing stabilizer. Endotoxin levels have been tested and do not exceed <2.5 EU/mL
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Antigen targeting to antigen-presenting cells (APCs) via specific receptors has been used to study vaccination strategies to induce effective antigen-specific T cell responses. Ovalbumin is widely used as a model antigen for the characterization of antigen uptake, processing, and presentation in mouse APCs. Especially the induction of $CD8^+$ T cell responses after targeting antigen via antigen uptake receptors to dendritic cells (DCs), commonly termed cross-priming, has raised major interest. For functional studies of antigen presentation ovalbumin T cell receptor-transgenic $CD4^+$ and $CD8^+$ T cells from DO11.10, OT-II and OT-I mouse strain are often used.[1–5]

The Ova Antigen Delivery Reagent has been developed for the *in vitro* targeting of ovalbumin to APCs, analysis of antigen uptake, and detection of antigen routes during antigen processing. It is a monoclonal anti-biotin antibody conjugated to ovalbumin and FITC. In combination with an appropriate biotinylated anti-receptor antibody any desired antigen uptake receptor can be targeted. This allows the functional characterization of new receptors on APCs for comparison with well-characterized ones, such as CD205 (DEC205) or DCIR2 (33D1). The Ova Antigen Delivery Reagent is also well suited for the analysis of antigen uptake and trafficking by fluorescent or confocal laser scanning microscopy.

1.2 Applications

The Ova Antigen Delivery Reagent is well suited for

- analysis of antigen-uptake by antigen-presenting cells, i.e., DCs, macrophages, and B cells
- analysis of antigen-processing and intracellular trafficking of endocytotic receptors
- research on cross-presentation of antigens
- research on protocols of antigen-delivery for DC vaccination

by using, for example, confocal laser scanning microscopy, flow cytometry, or downstream applications, such as CFSE-labeling, or ^{3}H-incorporation for the detection of T cell proliferation.

1.3 Reagent requirements

- Buffer: Prepare a sterile solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% fetal bovine serum (FBS), and 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).

  ▲ **Note:** Staining of some antigen uptake receptors, e.g., members of the c-type lectin family, require the presence of Ca^{2+} ions. Please use buffers recommended by the manufacturer for staining of those receptors with monoclonal antibodies.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) CD11c MicroBeads, mouse (# 130-052-001) or Pan DC MicroBeads, mouse (# 130-092-465) for the separation of $CD11c^+$ mouse dendritic cells.
- (Optional) $CD4^+$ T Cell Isolation Kit, mouse (# 130-090-860) for the separation of untouched mouse T helper cells.
- (Optional) $CD8a^+$ T Cell Isolation Kit, mouse (# 130-090-859) for the separation of untouched cytotoxic mouse T cells.
- (Optional) CD205 (DEC205)-Biotin, mouse (# 130-092-468). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Cell culture medium, e.g. RPMI 1640 (# 130-091-440) containing 5% mouse serum.

2. Protocol

2.1 Sample preparation

▲ If $CD11c^+$ DCs from mouse spleen are used as target cells single-cell suspensions have to be prepared by enzymatic disaggregation with Collagenase D for highest recovery and purity. Protocols which rely only on mechanical disruption are not recommended.

For preparing a single-cell suspension please refer to our gentleMACS™ Protocol "Preparation of single-cell suspensions from mouse spleen with Collagenase D treatment".

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

140-002-722.02

For details see the protocols section at www.miltenyibiotec.com/protocols.

2.2 *In vitro* targeting using Ova Antigen Delivery Reagent

▲ Method in brief: Isolated antigen-presenting cells are labeled with a biotin-conjugated antibody, which is specific for a cell surface receptor of interest. Cells are then labeled with the Ova Antigen Delivery Reagent for receptor-mediated targeting of ovalbumin to these cells.

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For accurate analysis of antigen targeting a negative control sample should always be included, for example, by omitting the antigen uptake receptor–specific biotin-conjugated antibody.

▲ Positive control, such as a sample labeled with CD205 (DEC-205)-Biotin may also be included. For details refer to the respective data sheet.

▲ Work under sterile conditions throughout the whole experiment.

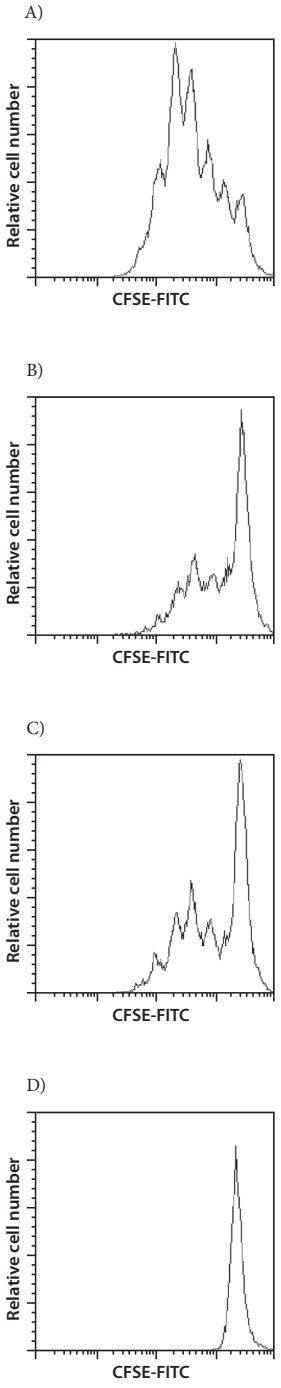
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.
3. Label cells with biotinylated antibody at time and titer recommended by the manfacturer. Typically, labeling for 10 minutes is sufficient.
   ▲ **Note:** The biotinylated antibody should be used at its optimal titer, i.e., with optimal labeling intensity and no background labeling.
4. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge for 10 minutes at 300×g and 4 °C.
   ▲ **Note:** The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.
5. Resuspend up to 10^6 nucleated cells per 100 µL of cold buffer.
6. Add 10 µL of the Ova Antigen Delivery Reagent.
7. Mix well and incubate for 10 minutes on ice.
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
8. Wash cells by adding 1–2 mL of buffer or medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. For subsequent analysis by flow cytometry resuspend cell pellet in a suitable amount of buffer or for culture in medium.

   Recommendation: The ovalbumin antigen presentation can be detected by proliferation of ovalbumin-specific transgenic T cells derived from OT-I, OT-II, or DO11.10 mouse strains.

   For more information about downstream analysis of antigen presentation refer to www.miltenyibiotec.com/protocols.

3. Examples of using the Ova Antigen Delivery Reagent

Dendritic cells (DCs) were isolated using CD11c MicroBeads, mouse. Ovalbumin was targeted to DCs via DCIR2 (33D1) (A), CD205 (DEC205) (B), or CD36 (C), using biotin-conjugated antibodies against the respective antigen uptake receptors or for control without a biotin-conjugated antibody (D) in combination with the Ova Antigen Delivery Reagent. Cells were cultured in the presence of Pam3CysSK4 for 24 h that induces DC maturation via toll-like receptors (TLR). Subsequently, cells were cultured with CFSE-labeled $CD4^+$ T cells from OT-II mice at a ratio of 1:5 (DC : T cell). After three days of proliferation T cells were analyzed by flow cytometry.



4. References

1. Bonifaz, L. *et al.* (2002) Efficient Targeting of Protein Antigen to the Dendritic Cell Receptor DEC-205 in the Steady State Leads to Antigen Presentation on Major Histocompatibility Complex Class I Products and Peripheral $CD8^+$ T Cell Tolerance. J. Exp. Med. 196: 1627–1638.
2. Dudziak, D. *et al.* (2007) Differential Antigen Processing by Dendritic Cell Subsets in Vivo. Science 315: 107–111.
3. Mouriès, J. *et al.* (2008) Plasmacytoid dendritic cells efficiently cross-prime naive T cells in vivo after TLR activation. Blood 112: 3713–3722.
4. Caminschi, I. *et al.* (2008) The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. Blood 112: 3264–3273.
5. Sancho, D. *et al.* (2008) Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. J. Clin. Invest. 118: 2098–2110.

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

gentleMACS and the Miltenyi Biotec logo and are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.