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## 1. Description

<b>Components</b>	200 µL Ova Antigen Delivery Reagent: monoclonal Anti-Biotin antibodies (isotype: mouse IgG1) conjugated to ovalbumin (Ova) and fluorescein isothiocyanate (FITC).
<b>Capacity</b>	20 tests or up to 2×10 <sup>7</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer. Endotoxin levels have been tested and do not exceed <2.5 EU/mL
<b>Storage</b>	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<sup>+</sup> 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<sup>+</sup> and CD8<sup>+</sup> T cells from DO11.10, OT-II and OT-I mouse strain are often used.<sup>1–5</sup>

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 <sup>3</sup>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<sup>2+</sup> 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<sup>+</sup> mouse dendritic cells.
- (Optional) CD4<sup>+</sup> T Cell Isolation Kit, mouse (# 130-090-860) for the separation of untouched mouse T helper cells.
- (Optional) CD8a<sup>+</sup> 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](http://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<sup>+</sup> 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”.

For details see the protocols section at [www.miltenyibiotec.com/protocols](http://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  $2 \times 10^6$  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  $300 \times g$  for 10 minutes at  $4^\circ C$ . Aspirate supernatant completely.
3. Label cells with biotinylated antibody at time and titer recommended by the manufacturer. 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  $300 \times g$  and  $4^\circ C$ .
 

▲ 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  $\mu L$  of cold buffer.
6. Add 10  $\mu L$  of the Ova Antigen Delivery Reagent.
7. Mix well and incubate for 10 minutes on ice.
 

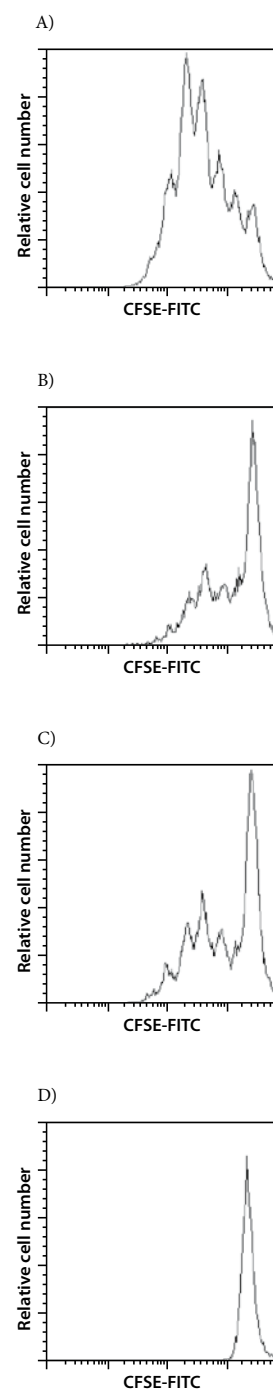
▲ Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
8. Wash cells by adding 1–2 mL of buffer or medium and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
9. For subsequent analysis by flow cytometry resuspend cell pellet 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](http://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<sup>+</sup> 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.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

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