



Miltenyi Biotec

## Contents

### 1. Description

- 1.1 Background information
- 1.2 Applications
- 1.3 Recommended antibody dilution
- 1.4 Reagent requirements

### 2. General protocol for immunofluorescent staining

### 3. Examples of immunofluorescent staining with Anti-HLA-DR antibodies

## 1. Description

|                       |   |
|-----------------------|---|
| <b>Components</b>     | 1 mL Anti-HLA-DR antibodies, human: monoclonal anti-HLA-DR antibodies conjugated to VioBlue®, fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or peridinin chlorophyll protein (PerCP). |
| <b>Clone</b>          | AC122 (isotype: mouse IgG2a).   |
| <b>Capacity</b>       | 100 tests or up to 10 <sup>9</sup> total cells.   |
| <b>Product format</b> | Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.   |
| <b>Storage</b>        | Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.  |

Cross-reactivity: The Anti-HLA-DR antibody has been tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

### 1.1 Background information

Anti-HLA-DR antibodies react with the human major histocompatibility (MHC) class II antigen HLA-DR. HLA-DR is constitutively expressed on professional antigen-presenting cells like dendritic cells, B cells, and monocytes/macrophages. Its expression is further up-regulated upon activation. On T cells, NK cells, hematopoietic precursor cells, and some epithelial cells the expression of HLA-DR is induced by cell activation.

### 1.2 Applications

- Identification and enumeration of HLA-DR<sup>+</sup> cells from PBMCs, cord blood, bone marrow, and tissues by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human antigen-presenting cells can be isolated by using, for example, Anti-HLA-DR MicroBeads, human (# 130-046-101).

# Anti-HLA-DR antibodies human

|                      |             |
|----------------------|-------------|
| Anti-HLA-DR-VioBlue® | 130-095-293 |
| Anti-HLA-DR-FITC     | 130-095-295 |
| Anti-HLA-DR-PE       | 130-095-298 |
| Anti-HLA-DR-APC      | 130-095-297 |
| Anti-HLA-DR-PerCP    | 130-095-291 |

- Study of expression level of HLA-DR on professional APC-activated T cells or epithelial cells.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

| Anti-HLA-DR conjugate                 | VioBlue | FITC  | PE   | APC  | PerCP |
|---------------------------------------|---------|-------|------|------|-------|
| Flow cytometry <sup>a</sup>           |         |       |      |      |       |
| - In general                          | 1:11    | 1:11  | 1:11 | 1:11 | 1:11  |
| - Formaldehyde-fixed cells            | 1:11    | 1:11  | 1:11 | 1:11 | 1:11  |
| - Anti-HLA-DR MicroBead-labeled cells | n. r.   | n. r. | 1:11 | 1:11 | n. r. |

a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100 µL of buffer. n. r.: not recommended

### 1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD14-FITC (# 130-080-701) or CD14-PE (# 130-091-242). For more information about antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Mouse IgG2a-VioBlue (# 130-094-671), Mouse IgG2a-FITC (# 130-091-837), Mouse IgG2a-PE (# 130-091-835), Mouse IgG2a-APC (# 130-091-836), or Mouse IgG2a-PerCP (# 130-094-967) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

## 2. General protocol for immunofluorescent staining

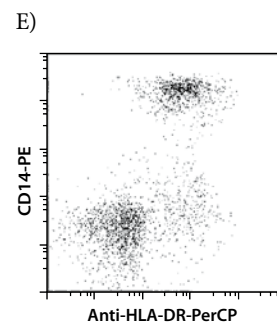
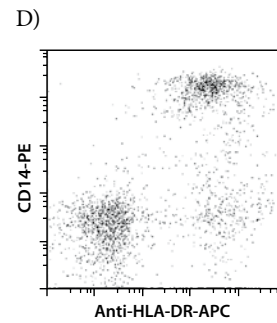
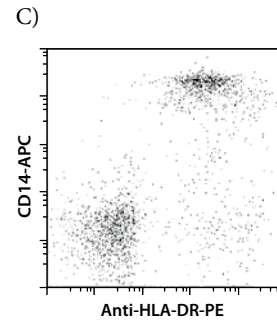
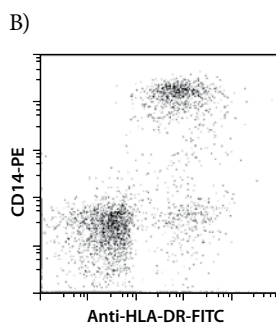
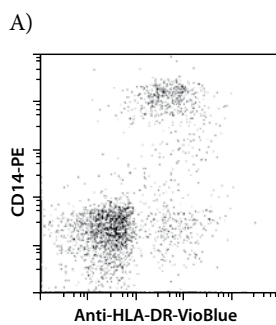
▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

140 002 893 01

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add 10 μL of the Anti-HLA-DR antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).  
▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

### 3. Examples of immunofluorescent staining with Anti-HLA-DR antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-HLA-DR antibodies conjugated to VioBlue (A), FITC (B), PE (C), APC (D), or PerCP (E) as well as with CD14-PE (# 130-091-242) or CD14-APC (# 130-091-243) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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

#### Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

#### Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, MACSQuant, and VioBlue are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2010 Miltenyi Biotec GmbH. All rights reserved.