

# Anti-DO11.10 TCR antibodies

## mouse

Anti-DO11.10 TCR-VioBlue®	130-095-313
Anti-DO11.10 TCR-FITC	130-095-306
Anti-DO11.10 TCR-PE	130-095-309
Anti-DO11.10 TCR-APC	130-095-311
Anti-DO11.10 TCR-Biotin	130-095-304
Anti-DO11.10 TCR pure – functional grade	130-095-379

### 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-DO11.10 TCR antibodies
4. References

## 1. Description

<b>Components</b>	1 mL Anti-DO11.10 TCR antibodies, mouse: monoclonal Anti-DO11.10 TCR antibodies conjugated to VioBlue®, fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin.  0.5 mL Anti-DO10.11 TCR pure – functional grade antibody, mouse.
<b>Clone</b>	KJ1-26 (isotype: mouse IgG2a).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.  The functional grade antibody is supplied at a concentration of 1 mg/mL.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.  Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/μg of protein.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

*The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.*

### 1.1 Background information

The KJ1-26 monoclonal antibody reacts with the T cell receptor (TCR) expressed on lymphocytes of the DO11.10 transgenic mouse<sup>1</sup> and the TCR of the BALB/c-derived DO11.10 and DO11.10.24 T cell hybridoma.<sup>2,3</sup> The DO11.10 TCR is specific for the chicken ovalbumin (OVA) peptide (323-339) in the context of I-A[d] major histocompatibility (MHC) molecules. As reported, transgenic DO11.10 T cells also recognize OVA-peptide from jungle fowl and turkey in the presence of A20-I.11, the H-2<sup>d</sup>-bearing, antigen-presenting B cell lymphoma.<sup>2</sup>

### 1.2 Applications

- The DO11.10 mouse model is a valuable tool for studies of T cell immigration, immunoregulation, development, activation, and function.<sup>1,4-6</sup>
- The KJ1-26 monoclonal antibody was shown to block the antigen responses of the DO11.10 T cell hybridoma *in vitro*.<sup>2,3</sup> In numerous experiments transgenic T cells of DO11.10 mice were isolated, labeled with fluorescent cell staining dyes, and transferred to BALB/c background mice. After vaccination experiments with OVA, transgenic T cells are harvested and analyzed for proliferation by flow cytometry. A special protocol for DO11.10 T cell enrichment for the analysis of very low concentrations of DO11.10 T cells is available.
- Conjugates of KJ1-26 have been reported to be used for flow cytometry, blocking, ELISA, immunohistochemistry, and immunoprecipitation.

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-DO11.10 TCR conjugate	VioBlue	FITC	PE	APC	Biotin
Flow cytometry <sup>a</sup>					
- In general	1:11	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11	1:11	1:11

- a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100 μL of buffer.  
b) For optimal results, cells must be stained prior to fixation.

### 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 mouse serum albumin, mouse 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, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue (# 130-094-669), Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-DO11.10 TCR-Biotin.

- (Optional) CD4-PE (# 130-091-607) or CD4-APC (# 130-091-611) and CD3 $\epsilon$ -FITC (# 130-092-962), CD3 $\epsilon$ -PE (# 130-092-976), or CD3 $\epsilon$ -APC (# 130-092-977). For more information about antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (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.

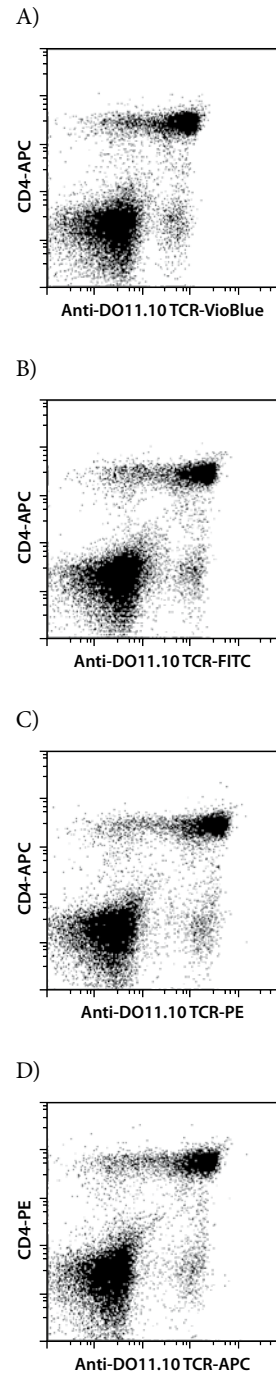
## 2. General protocol for immunofluorescent staining

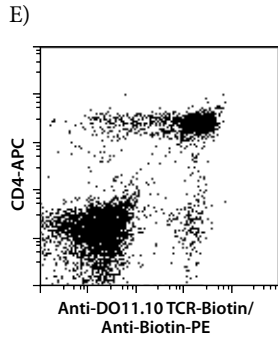
▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for  $2 \times 10^7$  nucleated cells, use twice the volume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

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 10  $\mu$ L of the Anti-DO11.10 TCR antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-DO11.10 TCR-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of anti-biotin antibody (Anti-Biotin-VioBlue, Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

## 3. Examples of immunofluorescent staining with Anti-DO11.10 TCR antibodies

Spleen cells from DO11.10 transgenic mice were stained with Anti-DO11.10 TCR antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) as well as with CD4-PE (# 130-091-607) or CD4-APC (# 130-091-611) and analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. Cells labeled with Anti-DO11.10 TCR-Biotin (E) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD4-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





#### 4. References

1. Murphy, K. M. *et al.* (1990) Induction by antigen of intrathymic apoptosis of CD4<sup>+</sup>CD8<sup>+</sup> TCR<sup>lo</sup> thymocytes *in vivo*. *Science* 250: 1720–1723.
2. Haskins, K. *et al.* (1983) The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157: 1149–1169.
3. Marrack, P. *et al.* (1983) The major histocompatibility complex-restricted antigen receptor on T cells. IV. An anti-idiotypic antibody predicts both antigen and I-specificity. *J. Exp. Med.* 158: 1635–1646.
4. Egan, R. M. *et al.* (1996) Peptide-specific T cell clonal expansion *in vivo* following immunization in the eye, an immune-privileged site. *J. Immunol.* 157: 2262–2271.
5. Garside P. *et al.* (1998) Visualization of specific B and T lymphocyte interactions in the lymph node. *Science* 281: 96–99.

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.