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Product datasheet for CL072R

MHC Class II I-Ap Mouse Monoclonal Antibody [Clone ID: 7-16.17]

Product data:

Product Type:	Primary Antibodies
Clone Name:	7-16.17
Applications:	FC
Recommended Dilution:	Flow cytometry (protocol see below). Cytotoxic assay.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	B10.p
Specificity:	CL072P is specific for Mouse-I-Ap,k,q,r,s,b. This antibody is a cytotoxic antibody which defines a public I-A antigen. Using recombinant strains, reactivity against the b haplotype has been localized to the Ab subregion. The antibody can be used to quantitate or eliminate I-A bearing cells for precipitating I-A antigen.
Formulation:	PBS containing 0.02% sodium azide (NaN3) as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml Label: PE State: Liquid Ig fraction Label: R - Phycoerythrin
Concentration:	lot specific
Purification:	Protein G chromatography
Conjugation:	PE
Storage:	Store the antibody undiluted at 2-8°C. **DO NOT FREEZE!**
Stability:	Shelf life: one year from despatch.

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Note:

Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 0.2-1.0 µg of antibody.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-mouse IgG) at 1/500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Strain Distribution by Flow Cytometry Analysis:

Procedure: As above
Antibody Concentration: 0.5 µg/10e6 cells
Strains Tested: see picture below.
For a more detailed strain distribution - see reference 1.