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- Trockeneiszuschlag
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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

FITC Anti-Mouse Ia^k Alloantiserum Anti-Human B Cell Serum

CL8701F

LOT: 510

DESCRIPTION:

Cedarlane's anti-mouse Ia alloantiserum is a broadly reactive Ia^k antiserum. The antiserum is prepared by immunizing A.TH mice with A.TL splenocytes. These two strains are congenic, differing at the I region of the H-2 complex but identical at the K and D regions. The two strains are characterized by the following H-2 haplotypes:

				I							
				K	A	B	E	C	S	G	D
A.TH	s	s	s	s	s	s	s	s	s	d	(K ^S I ^S D ^d)
A.TL	s	k	k	k	k	k	k	k	k	d	(K ^S I ^k D ^d)

The I region gene products (Ia antigens) potentially detected by this antiserum are those controlled by the following subregions: A^k, B^k, E^k, C^k i.e. it is a broadly reactive anti-Ia^k antiserum. It detects each of the following public and private specificities: Ia.1,2,3,7,15,19 and 22. As a result, this antiserum crossreacts with all standard haplotypes (i.e. H-2^{b,d,k,p,q,r}) but does not crossreact with H-2^s.

The Ia antigens are expressed as cell surface antigens on lymphocyte subpopulations and macrophages and perhaps other non-lymphocytic cells. This fluorescein conjugated antibody can be used to localize or quantitate cells bearing the appropriate Ia antigens by fluorescence microscopy or cell sorter analysis. Since the A.TH anti-A.TL antiserum is cytotoxic, treatment of immunologically competent cell populations with this antiserum plus complement can quantitate cells bearing the corresponding Ia antigen or eliminate these cells from the population for functional studies.

Ia Antigens on Human B Lymphocytes:

A.TH anti-A.TL antiserum is strongly cytotoxic to human B lymphocytes but is not cytotoxic to human T-lymphocytes. This antiserum appears to recognize a determinant present on both mouse Ia and human HLA-DR antigens. The antiserum reacts with B lymphocytes of all individuals tested by Cedarlane, although there is one report that B cells of some individuals are not reactive.

For more information or to place an order please contact...

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In addition to human peripheral blood B lymphocytes, this antiserum reacts with chronic lymphatic leukemia (CLL) cells and at least a portion of monocytes. As a cytotoxic antibody, it can be used with complement for the enumeration or elimination of B cells, and is particularly well-suited as a positive control serum for B cell typing. The antiserum can also be used for biochemical studies of HLA-DR molecules.

PRESENTATION:

FITC conjugated purified antibody in 0.05 M carbonate buffer, pH 9.6. No preservatives added.

FORMAT: 0.5 ml, lyophilized

STORAGE/STABILITY:

Store at -20°C before reconstitution. Reconstitute with 0.5 ml of distilled water. Agitate until all of the lyophilized material is dissolved. Aliquot and freeze the unused portion in volumes appropriate for single usage. Do not dilute prior to freezing. Store the frozen aliquots at -70°C or below. Avoid repeated freezing and thawing cycles as it will reduce the titer.

STERILITY:

This antiserum is not sold as sterile. Filtration may result in severe loss of activity. If desired, sodium azide (0.02% final concentration) may be added as a preservative.

RECOMMENDED METHOD FOR STAINING MOUSE LYMPHOCYTES WITH FITC - ANTI-Ia ANTIBODY:

Note: All procedures should be carried out on ice.

1. Prepare a cell suspension from the appropriate tissue in Dulbecco's phosphate buffered saline containing 0.3% bovine serum albumin plus 0.1% sodium azide (PBS-BSA). Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte-M density cell separation medium. After washing, adjust the cell concentration to 2×10^7 cells/ml in PBS-BSA.
2. Add 50 μl of cell suspension to the appropriate number of wells in a micro-titer round bottom plate.
3. Add 50 μl of appropriately diluted antibody (dilutions in PBS-BSA) to each well and mix.
4. Incubate for one hour on ice.
5. Pellet cells by centrifugation (500 x g for 3 minutes) and discard the supernatant. Wash x 2 with 100 μl of PBS-BSA. Remove supernatant.
6. For Fluorescent Microscopy: Resuspend in 50 μl of PBS-BSA. Place one drop on a microscope slide and cover with a cover glass. Score stained vs unstained cells in a fluorescent microscope.

For Cell Sorter Analysis: Resuspend to the appropriate cell concentration and proceed as usual.

Note: Prior to lyophilization, this product was clarified by centrifugation at 10000 x g for 10 minutes. If aggregates appear after prolonged storage of the reconstituted product, then centrifugation is recommended.

SPECIAL NOTE: It is reported that azides react with lead and copper in plumbing to form compounds that may detonate on percussion. Please flush drains with large volumes of water when disposing of solutions containing sodium azide.

LOT SPECIFICATIONS:

Lot No: 510

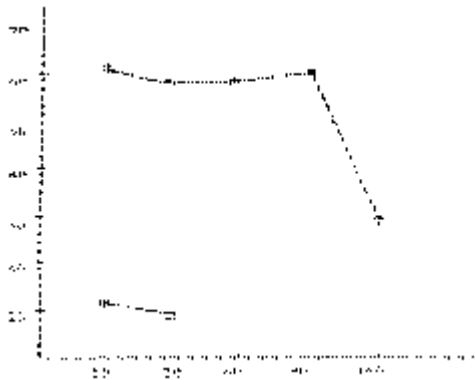
Protein Concentration: 43.7 ± 4.9 mg/ml.

Recommended Working Dilution (final): 1:20

Fluorescent Microscopy Analysis:

Titration

Method: As described above



Reciprocal of Final Antibody Dilution

“Enriched splenic B-cells” prepared by treatment of Lympholyte-M purified spleen lymphocytes with Cedarlane Monoclonal Anti-Thy 1.2 Antibody plus complement, followed by removal of dead cells on Lympholyte-M.

Tissue Distribution:

Method: As described above

Cell Donor: A.TL

Antibody Dilution Used: 1:20

<u>Cell Source</u>	<u>% Fluorescent Stained Cells</u>
Enriched Splenic B Cells	52%
Lymph Node	18%
Thymus	3%

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