Technical Data Sheet

Purified Rat Anti-Mouse CD4

Product Information

Material Number: 550278
Alternate Name: Cd4; CD4 antigen, L3T4; Ly-4; T-cell surface antigen T4/Leu-3
Size: 1 mL
Concentration: 62.5 µg/ml
Clone: H129.19
Immunogen: A.TH mouse CTL clone A15.1.17
Isotype: Rat (LOU) IgG2a, κ
Reactivity: Mouse
Storage Buffer: Aqueous buffered solution containing BSA, goat serum, and ≤0.09% sodium azide.

Description

The H129.19 monoclonal antibody specifically binds to the CD4 (L3T4) differentiation antigen expressed on thymocytes, a subpopulation of mature T lymphocytes (i.e., MHC class II-restricted T cells, including most T helper cells), and a subset of NK-T cells of all mouse strains tested. CD4 has also been detected on pluripotent hematopoietic stem cells, bone marrow myeloid precursors, intrathymic lymphoid precursors, and a subset of splenic dendritic cells. CD4 is expressed on the plasma membrane of mouse egg cells and is involved in adhesion of the egg to MHC class II-bearing sperm. CD4 is an antigen coreceptor on the T-cell surface which interacts with MHC class II molecules on antigen-presentation cells. It participates in T-cell activation through its association with the T-cell receptor complex and protein tyrosine Lck. The H129.19 antibody blocks binding of the anti-mouse CD4 antibodies GK1.5 and RM4-5, but not RM4-4 antibody. mAb H129.19 inhibits various responses of T helper cells to antigenic or mitogenic stimuli.

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

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<tr>
<th>Application</th>
<th>Routine Status</th>
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<tbody>
<tr>
<td>Flow cytometry</td>
<td>Routinely Tested</td>
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<tr>
<td>Immunohistochemistry-frozen</td>
<td>Tested During Development</td>
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<tr>
<td>Immunohistochemistry-zinc-fixed</td>
<td>Tested During Development</td>
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<tr>
<td>Immunoprecipitation</td>
<td>Reported</td>
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<tr>
<td>Blocking</td>
<td>Reported</td>
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<tr>
<td>Depletion</td>
<td>Reported</td>
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<tr>
<td>Immunohistochemistry-formalin</td>
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Recommended Assay Procedure:

Immunohistochemistry: The H129.19 antibody is recommended to test for immunohistochemical staining of acetone-fixed frozen sections and zinc-fixed paraffin sections. Tissues tested were mouse spleen and thymus. The antibody stains the membranes of thymocytes and a subpopulation of mature T lymphocytes that are MHC class II restricted. The isotype control recommended for use with this antibody is purified rat IgG2a (Cat. No. 559073). For optimal indirect immunohistochemical staining, the H129.19 antibody should be titrated (1:10 to 1:50 dilution) and visualized via a three-step staining procedure in combination with biotinylated polyclonal anti-rat Igis (multiple adsorbed) (Cat. No. 559286) as the secondary.

Immunohistochemical staining of CD4+ T lymphocytes.

Frozen sections of normal mouse spleen were reacted with H129.19 antibody. CD4+ T lymphocytes can be identified by the intense brown labeling of their cell surface membranes. Amplification 20X.
antibody and Streptavidin-HRP (Cat. No. 550946) together with the DAB detection system (Cat. No. 550880). More conveniently, the anti-rat Ig HRP detection kit (Cat. No. 551013) that contains the biotinylated secondary antibody, antibody diluent, streptavidin-HRP and DAB substrate can be used for staining. A detailed protocol of the immunohistochemical procedure is available on our website at wwwbdbiosciencescom/support/resources. The clone H129.19 is not recommended for formalin fixed paraffin embedded sections.

**Suggested Companion Products**

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<th>Catalog Number</th>
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<th>Size</th>
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</thead>
<tbody>
<tr>
<td>559073</td>
<td>Purified Rat IgG2a x Isotype Control</td>
<td>0.25 mg</td>
<td>R35-95</td>
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<tr>
<td>559286</td>
<td>Biotin Goat Anti-Rat Ig</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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<tr>
<td>550880</td>
<td>DAB Substrate Kit</td>
<td>500 Tests</td>
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<tr>
<td>551013</td>
<td>Anti-Rat Ig HRP Detection Kit</td>
<td>200 Tests</td>
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<tr>
<td>550946</td>
<td>Streptavidin HRP</td>
<td>50 mL</td>
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**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. An isotype control should be used at the same concentration as the antibody of interest.
5. This antibody has been developed for the immunohistochemistry application. However, a routine immunohistochemistry test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
6. Please refer to wwwbdbiosciencescom/pharmingen/protocols for technical protocols.

**References**

BD Biosciences Pharmingen. Unpublished results. (Clone-specific: Immunohistochemistry)