Technical Data Sheet

Purified Rat Anti-Mouse CD40

Product Information

Material Number: 550285
Size: 1.0 ml
Concentration: 125 µg/ml
Clone: 3/23
Immunogen: Mouse CD40 Recombinant Protein
Isotype: Rat (LOU) IgG2a, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing BSA, goat serum, and ≤0.09% sodium azide.

Description

The 3/23 clone reacts with CD40, a 40-50 kDa glycoprotein expressed on B lymphocytes and other antigen-presenting cells. CD40 has been reported to be transiently expressed on activated CD4+ and CD8+ T cells and in some mouse strains, the 3/23 mAb has been reported to react with 5-10% of T lymphocytes in adult mouse, but not neonatal, spleen. CD40 plays a key role in B-cell growth and differentiation where interactions of CD40 with its ligand, CD154, are involved in the initiation, effector, and memory stages of cell-mediated immune responses. In addition, CD40 has been reported to be involved with the triggering of NK cells and NK-T cells. Ligation of CD40 with the 3/23 antibody has been reported to induce splenic B cells to express the costimulatory molecule CD86 (B7-2). In addition, although the 3/23 antibody by itself is a weak B-cell mitogen, it has been reported to synergize markedly with mitogenic anti-IgM, anti-IgD mAb or IL-4 to promote B-cell proliferation.

Immunohistochemical staining of CD40. Frozen sections of normal mouse thymus was reacted with the 3/23 antibody. B lymphocytes and dendritic cells can be identified by the brown labeling of their cell surface membranes. Amplification 20x.

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

<table>
<thead>
<tr>
<th>Flow cytometry</th>
<th>Routinely Tested</th>
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</thead>
<tbody>
<tr>
<td>Immunohistochemistry-frozen</td>
<td>Tested During Development</td>
</tr>
<tr>
<td>Immunohistochemistry-zinc-fixed</td>
<td>Tested During Development</td>
</tr>
<tr>
<td>Immunohistochemistry-formalin (antigen retrieval required)</td>
<td>Not Recommended</td>
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</tbody>
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Recommended Assay Procedure:

Immunohistochemistry: Clone 3/23 is recommended to test for immunohistochemical staining on acetone-fixed frozen sections or zinc-fixed paraffin sections of mouse spleen or thymus. **IHC of formalin-fixed paraffin embedded sections is not recommended.** The isotype control recommended for use with this antibody is purified rat IgG2a (Cat. No. 559073). For optimal indirect immunohistochemical staining, the 3/23 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 Ig (multiple adsorbed) (Cat. No. 559286) as the secondary 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) can be used which contains the biotinylated secondary antibody, the antibody diluent, streptavidin-HRP and a DAB substrate for use in the staining procedure.
Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>559073</td>
<td>Purified Rat IgG2a κ 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>550946</td>
<td>Streptavidin HRP</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>
<td>(none)</td>
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</table>

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. 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.
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.

References


Parry SL, Hasbold J, Holman M, Klaus GG. Hypercross-linking surface IgM or IgD receptors on mature B cells induces apoptosis that is reversed by costimulation with IL-4 and anti-CD40. *J Immunol.* 1994; 152(6):2821-2829. (Biology)