Purified Mouse Anti-Human CD171

Product Information

Material Number: 554273
Alternate Name: L1 Neurite Cell Adhesion Molecule; N-CAM-L1; LICAM; CAML1; MIC5
Size: 0.1 mg
Concentration: 0.5 mg/ml
Clone: 5G3
Immunogen: Human SK-N-AS Neuroblastoma Cell Line
Isotype: Mouse IgG2a
Reactivity: QC Testing: Human
Target MW: 215/200 kDa
Workshop: VII 70700
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Neurite adhesion molecule L1 has been implicated in neuron-neuron and neuron-Schwann cell adhesion in vertebrates. L1-like molecules, found in mouse, rat, chicken, and human, promote axonal elongation and may also play a role in regeneration of axons after injury. Molecular cloning data suggest 87% amino acid identity between mouse and human L1 molecules. 5G3 antigen (Ag), originally defined by monoclonal antibody 5G3, is considered to be the human homologue of mouse L1. The 5G3 antibody was developed against a human neuroblastoma cell line to use as a probe for the elucidating the biological characteristics of neuroblastoma. 5G3 specifically recognizes a neuroblastoma target glycoprotein antigen of 215 kDa and its 200 kDa precursor. The 215 kDa molecule is expressed on the cell surface; whereas the 200 kDa precursor is shed from the cell surface. The 215 and 200 kDa species also differ in their posttranslational modification patterns. The 5G3 antibody has been used as a marker for neuroblastoma, and to purify 5G3 Ag from normal adult human brain.

The antibody recognizes human L1 on human neuroblastoma cell lines and tissues. Reactivity has been tested on a variety of malignant and normal tissues. Squamous lung, squamous skin, and osteogenic sarcoma cell lines were positive, as were two out of eight melanoma cell lines tested. A variety of other cell lines and tumor tissues tested negative. 5G3 did not react with either T or B lymphoblastoid cell lines or a fibroblast cell line. Among all the normal tissues tested, mAb 5G3 reacted only with cerebellum.

The molecular masses observed using mAb 5G3 may vary among immunoprecipitation isolates. In normal human cerebellum, 5G3 Ag migrated as a 190/200 kDa doublet, 140 kDa band with minor bands at 80 and 65 kDa. 5G3 Ag isolated from SK-N-AS cells migrates as 200 to 215 kDa bands, or as a diffuse band ranging from 200 to 215 kDa. Additional bands have been described at 140 to 150 kDa in SK-N-AS cells. Only the 200 kDa band has been detected in culture media from SK-N-AS cells.

Flow cytometric analysis of CD171 expression on human melanoma cells. M21 human melanoma cells were stained with either Purified Mouse IgG2a, κ Isotype Control (Cat. No. 555571; grey line histogram) or Purified Mouse Anti-Human CD171 (Cat. No. 554273, black line histogram). Secondary staining was carried out with FITC Goat Anti-Mouse IgG(μ)G (Cat. No. 555988). Fluorescence histograms were derived from gated events with the side and forward light scatter characteristics of viable M21 cells.

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>Method</th>
<th>Status</th>
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<tbody>
<tr>
<td>Flow cytometry</td>
<td>Routinely Tested</td>
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<tr>
<td>Immunoprecipitation</td>
<td>Reported</td>
</tr>
<tr>
<td>Immunohistochemistry-frozen</td>
<td>Reported</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>Reported</td>
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Recommended Assay Procedure:
Clone 5G3 can be used for western blot and IP analysis. The molecular masses observed using mAb 5G3 may vary among immunoprecipitation isolates. For western blot assay conditions, please review the references below.

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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</thead>
<tbody>
<tr>
<td>555571</td>
<td>Purified Mouse IgG2a, κ Isotype Control</td>
<td>0.1 mg</td>
<td>G155-178</td>
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<tr>
<td>555988</td>
<td>FITC Goat Anti-Mouse IgG/IgM</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
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</tbody>
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Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References
Rathjen FG, Schachner M. Immunocytopathological and biochemical characterization of a new neuronal cell surface component L1 which is involved in cell adhesion. EMBO J. 1984; 3(1):1-10. (Biology)