**Product Information**

**Material Number:** 565737  
**Alternate Name:** L1 Neurite Cell Adhesion Molecule; N-CAM-L1; LICAM; CAML1; MIC5  
**Size:** 25 Tests  
**Vol. per Test:** 5 µl  
**Clone:** 5G3  
**Immunogen:** Human SK-N-AS Neuroblastoma Cell Line  
**Isotype:** Mouse IgG2a  
**Reactivity:** QC Testing: Human  
**Workshop:** VII 70700  
**Storage Buffer:** Aqueous buffered solution containing BSA and ≤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.

The antibody was conjugated to BD Horizon BUV395 which is part of the BD Horizon Brilliant™ Ultraviolet family of dyes. This dye is optimal for multicolor flow cytometry because it has little to no spillover into other detectors. With an Ex Max at 348 nm and an Em Max at 395 nm, BD Horizon BUV395 can be excited with a 355 nm laser and detected with a 379/28 filter.

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**Flow cytometric analysis of CD171 expression on human M21 melanoma cells.** Cells from the human M21 melanoma cell line were stained with either BD Horizon™ BUV395 Mouse IgG2a, κ Isotype Control (Cat. No. 563809; dashed line histogram) or BD Horizon BUV395 Mouse Anti-Human CD171 antibody (Cat. No. 565736/565737; solid line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable M21 cells. Flow cytometric analysis was performed using a BD LSRSortessa™ Cell Analyzer System.

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565737 Rev. 1
Preparation and Storage
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with BD Horizon™ BUV395 under optimum conditions, and unconjugated antibody and free BD Horizon™ BUV395 were removed.

Application Notes

Application

| Flow cytometry | Routinely Tested |

Recommended Assay Procedure:
For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Catalog number 563794).

Suggested Companion Products

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<th>Name</th>
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<td>Stain Buffer (FBS)</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
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<td>563809</td>
<td>BUV395 Mouse IgG2a, κ Isotype Control</td>
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Product Notices
1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use $1 \times 10^6$ cells in a 100-µl experimental sample (a test).
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. An isotype control should be used at the same concentration as the antibody of interest.

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