Technical Data Sheet

BV421 Mouse Anti-Glucagon

Product Information

Material Number: 565891
Alternate Name: GLP1; GLP2; GRPP; GCG
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: U16-850
Immunogen: Human glucagon Recombinant Protein
Isotype: Mouse (BALB/c) IgG1, κ
Reactivity: Tested in Development: Mouse, Rat

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The U16-850 monoclonal antibody specifically binds to glucagon, a member of the secretin family of active peptides. Glucagon is an evolutionarily conserved peptide hormone that participates in the regulation of carbohydrate metabolism by counteracting the effects of insulin. Glucagon is produced by α cells in the islets of Langerhans of the pancreas. The CGC gene encodes the precursor molecule preproglucagon, which is cleaved to form proglucagon that in turn is cleaved to form at least four distinct peptides, including glucagon, in different tissues. Hypoglycemia causes the secretion of glucagon, which binds to the class B G-protein-coupled glucagon receptor that is mainly expressed in liver and kidney, causing reduced glycogenesis and glycolysis and increased glyconeogenesis and gluconeogenesis. The expression of glucagon can be used to monitor the pancreatic differentiation of pluripotent stem cells.

The antibody was conjugated to BD Horizon BV421 which is part of the BD Horizon Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (e.g., 450/50-nm filter). BD Horizon BV421 conjugates are very bright, often exhibiting a 10-fold improvement in brightness compared to Pacific Blue conjugates.

Flow cytometric analysis of Glucagon expression in a mouse pancreatic α cell line. Alpha TC1-6 cells (ATCC CRL-2934) were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were washed and then stained with either BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat. No. 562438, dotted-line histogram) or BD Horizon BV421 Mouse Anti-Glucagon (Cat. No. 565891, solid-line histogram). The fluorescence histogram was derived from gated events with the forward and side light-scatter characteristics of intact cells. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.

Immunohistofluorescent staining of Glucagon in human, mouse, and rat islets of Langerhans. Following antigen retrieval with BD Pharmingen™ Retrieval A Buffer (Cat. No. 550524), sections from formalin-fixed, paraffin-embedded human (top row), mouse (middle row), and rat (bottom row) pancreata were blocked using an Avidin/Biotin Blocking Kit (Vector Laboratories, Cat. No. SP-2001) as recommended by the manufacturer.

LEFT PANEL: These sections were then stained with BD Horizon BV421 Mouse Anti-Glucagon (Cat. No. 565891, pseudocolored red), and cell nuclei were counterstained with BD Pharmingen™ DRAQ5™ (Cat. No. 564902 pseudo-colored green).

RIGHT PANEL: These sections were then stained with BD Horizon BV421 Mouse Anti-Glucagon (Cat. No. 565891, pseudocolored red) and Alexa Fluor® 647 Mouse Anti-Insulin (Cat. No. 565689, pseudocolored green). The cell nuclei were counterstained with BD Pharmingen™ 7-AAD (Cat. No. 559925, pseudocolored blue, top and middle rows only). The typical localization of the α cells is seen: distributed throughout human islets and at the periphery in rodent islets.

The photographs were performed on a standard epifluorescence microscope. Original magnification, 20x.
**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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

**Application Notes**

**Application**

<table>
<thead>
<tr>
<th>Intracellular staining (flow cytometry)</th>
<th>Routinely Tested</th>
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</thead>
<tbody>
<tr>
<td>Immunofluorescence</td>
<td>Tested During Development</td>
</tr>
<tr>
<td>Immunohistochemistry-paraffin</td>
<td>Tested During Development</td>
</tr>
</tbody>
</table>

**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 (Cat. No. 563794).

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>554655</td>
<td>Fixation Buffer</td>
<td>100 mL</td>
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</tr>
<tr>
<td>558050</td>
<td>Perm Buffer III</td>
<td>125 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>562438</td>
<td>BV421 Mouse IgG1, k Isotype Control</td>
<td>50 µg</td>
<td>X40</td>
</tr>
<tr>
<td>550524</td>
<td>Retrievagen A (pH 6.0)</td>
<td>1000 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>564902</td>
<td>DRAQ5™</td>
<td>200 µL</td>
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<tr>
<td>565689</td>
<td>Alexa Fluor® 647 Mouse Anti-Insulin</td>
<td>25 µg</td>
<td>T56-706</td>
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<td>559925</td>
<td>7-AAD</td>
<td>2 mL</td>
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<tr>
<td>563794</td>
<td>Brilliant Stain Buffer</td>
<td>100 Tests</td>
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</tr>
</tbody>
</table>

**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
4. DRAQ5™ is a registered trademark of BioStatus Ltd.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
10. An isotype control should be used at the same concentration as the antibody of interest.

**References**


