Technical Data Sheet

BV421 Mouse Anti-Human CD3

Product Information

- **Material Number:** 562426
- **Alternate Name:** CD3ε; CD3E; T3E; TCRE; T-cell surface antigen T3/Leu-4 epsilon
- **Size:** 100 Tests
- **Vol. per Test:** 5 µl
- **Clone:** UCHT1
- **Immunogen:** Human infant thymocytes and peripheral blood lymphocytes from a Sézary Syndrome donor
- **Isotype:** Mouse (BALB/c) IgG1, κ
- **Reactivity:** QC Testing: Human
- **Workshop:** III 471
- **Storage Buffer:** Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The UCHT1 monoclonal antibody specifically binds to the human CD3ε-chain, a 20-kDa subunit of the CD3/T cell antigen receptor complex. CD3ε is expressed on 70-80% of normal human peripheral blood lymphocytes and 60-85% of thymocytes. Studies from the HLDA Workshop show that this antibody is mitogenic for CD3ε-positive cells when used in conjunction with costimulatory agents such as pokeweed mitogen or anti-CD28 antibody. CD3 plays a central role in signal transduction during antigen recognition. The UCHT1 antibody stains both surface and intracellular CD3ε unlike the other CD3 clone, HIT3a, that stains only extracellular CD3ε.

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 (eg, 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 CD3 expression on human peripheral blood lymphocytes (left panel). Human whole blood was stained with the BD Horizon™ BV421 Mouse anti-Human CD3 antibody (Cat. No. 562426/562427; solid line histogram) or with BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat. No. 562438; dashed line histogram). The erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD FACSCanto™ II Flow Cytometer System.

Immunohistofluorescent analysis of CD3 expression by cells within human tonsil (right panel). A human tonsil cryosection (5 µm) was fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), blocked with 5% goat serum and 1% BSA diluted in 1x PBS, and stained with BD Pharmingen™ Purified Mouse Anti-Human CD11c antibody (Cat. No. 565805) followed by BD Horizon™ BV480 Goat Anti-Mouse Ig second step antibody (Cat. No. 564877, pseudo-colored green). Sections were thoroughly washed, then stained with BD Horizon™ BV421 Mouse Anti-Human CD3 antibody (Cat. No. 562426/562427, pseudo-colored red) and Alexa Fluor® 488 Mouse Anti-Human CD19 antibody (Cat. No. 557697, pseudo-colored blue). Images were captured 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>Flow cytometry</th>
<th>Routinely Tested</th>
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</thead>
<tbody>
<tr>
<td>Immunofluorescence</td>
<td>Tested During Development</td>
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</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>
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<tbody>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
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<tr>
<td>555899</td>
<td>Lysing Buffer</td>
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<tr>
<td>562438</td>
<td>BV421 Mouse IgG1, k Isotype Control</td>
<td>50 µg</td>
<td>X40</td>
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<tr>
<td>562427</td>
<td>BV421 Mouse Anti-Human CD3</td>
<td>25 Tests</td>
<td>UCHT1</td>
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<tr>
<td>554655</td>
<td>Fixation Buffer</td>
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<tr>
<td>565805</td>
<td>Purified Mouse Anti-Human CD11c</td>
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<tr>
<td>564877</td>
<td>BV480 Goat Anti-Mouse Ig</td>
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<td>Polyclonal</td>
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<tr>
<td>557697</td>
<td>Alexa Fluor® 488 Mouse Anti-Human CD19</td>
<td>100 Tests</td>
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<td>349202</td>
<td>BD FACS™ Lysing Solution</td>
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<tr>
<td>562427</td>
<td>BD FACSTM Lysing Solution</td>
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<td>564907</td>
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<td>563794</td>
<td>DAPI Solution</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).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. BD Horizon Brilliant Violet 421 is covered by one or more of the following US patents: 8,158,444; 8,362,193; 8,575,303; 8,354,239.
8. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,158,444; 8,354,239.
9. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References