**Technical Data Sheet**

**BV421 Mouse Anti-Human CD3**

**Product Information**
- **Material Number:** 562877
- **Alternate Name:** CD3E; CD3-epsilon; T3E; TCRE
- **Size:** 50 Tests
- **Vol. per Test:** 5 µl
- **Clone:** SP34-2
- **Immunogen:** Purified Human CD3ε Protein
- **Isotype:** Mouse (BALB/c) IgG1, λ
- **Reactivity:** QC Testing: Rhesus, Cynomolgus, Baboon
  - Tested in Development: Human
- **Storage Buffer:** Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**
Clone SP34-2 is a mouse IgG1 isotype monoclonal antibody, descendant of SP34 (mouse IgG3), with the same specificity and reactivity pattern as the parent clone. It cross-reacts with a major subset of peripheral blood lymphocytes, but not monocytes or granulocytes, of baboon, and rhesus, cynomolgus, and pigtail macaque monkeys. The distribution on lymphocytes is similar to that observed with normal human donor lymphocytes with the majority of CD3-positive cells being negative when dual stained with antibodies to B or NK cells markers. SP34-2 is also capable of inducing cell proliferation on both human and non-human primate PBMC.

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.

**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**

| Flow cytometry | Routinely Tested |

---

**Flow cytometric analysis of CD3 expression on Rhesus peripheral blood lymphocytes.** Rhesus whole blood was stained with either a BD Horizon™ BV421 Mouse IgG1, κ Isotype Control (Cat. No. 562438; dashed line histogram) or with BD Horizon™ BV421 Mouse Anti-Human CD3 antibody (Cat. No. 562877; solid line histogram). Erythrocytes were lysed with BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.
Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes (including BD OptiBuild Brilliant reagents) 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. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>562438</td>
<td>BV421 Mouse IgG1, k Isotype Control</td>
<td>50 µg</td>
<td>X40</td>
</tr>
<tr>
<td>555899</td>
<td>Lysing Buffer</td>
<td>100 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>534656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>534657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>563794</td>
<td>Brilliant Stain Buffer</td>
<td>100 Tests</td>
<td>(none)</td>
</tr>
<tr>
<td>566349</td>
<td>Brilliant Stain Buffer</td>
<td>1000 Tests</td>
<td>(none)</td>
</tr>
<tr>
<td>566385</td>
<td>Brilliant Stain Buffer Plus</td>
<td>1000 Tests</td>
<td>(none)</td>
</tr>
</tbody>
</table>

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
8. 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.
9. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.

References

Conrad ML, Davis WC, Koop BF. TCR and CD3 antibody cross-reactivity in 44 species. Cytometry A. 2007; 71(11):925-933. (Biology)
Pessano S, Oettgen H, Bhan AK, Terhorst C. The T3/T cell receptor complex: antigenic distinction between the two 20-kd T3 (T3-delta and T3-epsilon) subunits. EMBO J. 1985; 4(2):337-344. (Immunogen: Activation, Dot Blot, Functional assay, Immunoprecipitation, Western blot)