**Product Information**

**Material Number:** 565567

**Alternate Name:** CD366; HAVCR2; TIM3; T cell immunoglobulin mucin-3; TIMD-3; KIM-3

**Size:** 25 Tests

**Vol. per Test:** 5 µl

**Clone:** 7D3

**Immunogen:** Human TIM-3

**Isotype:** Mouse IgG1, κ

**Reactivity:** QC Testing: Human

**Workshop:** X

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

**Description**

The 7D3 monoclonal antibody specifically binds to T cell immunoglobulin mucin 3 (TIM-3) which is also known as, CD366, or T-cell immunoglobulin and mucin domain-containing protein 3 (TIMD-3/TIM3). CD366 is encoded by the *HAVCR2* gene (Hepatitis A virus cellular receptor 2). CD366 is a type I transmembrane glycoprotein and belongs to the human TIM family (along with TIM-1 and TIM-4) within the immunoglobulin superfamily. CD366 is expressed on Th1, Tc1, Th17, Treg, NK T, and NK cells. CD366 is also expressed on dendritic cells, mast cells, monocytes, and macrophages. It is not expressed by Th2 and B cells. CD366 helps maintain peripheral immune tolerance and homeostasis. CD366 regulates macrophage activation and is a negative regulator of Th1 cell function. Crosslinking of cell surface CD366 by binding to Galectin-9 and/or phosphatidylserine appears to play an important role in either positively or negatively regulating leukocyte functions, such as cytokine production or the phagocytosis of apoptotic cells. CD366 may also be useful as an AML stem cell surface marker because it appears to be more highly expressed by AML leukemia stem cells than by normal bone marrow hematopoietic stem cells.

The antibody was conjugated to BD Horizon BV711 which is part of the BD Horizon Brilliant™ Violet family of dyes. This dye is a tandem fluorochrome of BD Horizon BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 711-nm. BD Horizon BV711 can be excited by the violet laser and detected in a filter used to detect Cy™5.5 / Alexa Fluor® 700-like dyes (eg, 712/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there may be moderate spillover into the Alexa Fluor® 700 and PerCP-Cy5.5 detectors. However, the spillover can be corrected through compensation as with any other dye combination.

**Multiparameter flow cytometric analysis of TIM-3 (CD366) expression on human peripheral blood leukocytes.** Human whole blood was stained with FITC Mouse Anti-Human CD56 antibody (Cat. No. 562794) and either BD Horizon™ BV711 Mouse IgG1 κ Isotype Control (Cat. No. 563044; Top Panels) or BD Horizon BV711 Mouse Anti-Human TIM-3 (CD366) antibody (Cat. No. 565566/565567; Bottom Panels). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202).

**Left Panels** - Two-parameter flow cytometric dot plots showing the correlated expression of TIM-3 (CD366) [or Ig Isotype control staining] versus side light-scatter (SSC-A) signals were derived from gated events with the forward and side light-scatter characteristics of intact leukocyte populations.

**Right Panels** - The two-color flow cytometric dot plots showing the correlated expression of TIM-3 (CD366) [or Ig Isotype control staining] versus CD56 were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes.

Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.
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™ BV711 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV711 were removed.

Application Notes

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<thead>
<tr>
<th>Application</th>
<th>Flow cytometry</th>
<th>Routinely Tested</th>
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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. 563794/566349).

Suggested Companion Products

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<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>
<td>500 mL</td>
<td>(none)</td>
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<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
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<tr>
<td>563794</td>
<td>Brilliant Stain Buffer</td>
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<td>563044</td>
<td>BV711 Mouse IgG1, k Isotype Control</td>
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<td>X40</td>
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<td>565566</td>
<td>BV711 Mouse Anti-Human TIM-3 (CD366)</td>
<td>100 Tests</td>
<td>7D3</td>
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<td>FITC Mouse anti-Human CD56</td>
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<tr>
<td>349202</td>
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<tr>
<td>555899</td>
<td>Lysing Buffer</td>
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<tr>
<td>566349</td>
<td>Brilliant Stain Buffer</td>
<td>1000 Tests</td>
<td>(none)</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 × 10⁶ 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. Alexa Fluor® is a registered 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. Cy is a trademark of GE Healthcare.
8. BD Horizon Brilliant Violet 711 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,575,303; 8,354,239.
9. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,104,673; 8,158,444; 8,575,303; 8,354,239.

References