Technical Data Sheet

Human CD4 Fluorochrome Evaluation Kit

Product Information

<table>
<thead>
<tr>
<th>Material Number:</th>
<th>566352</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size:</td>
<td>1 Each</td>
</tr>
<tr>
<td>Concentration:</td>
<td>50 µg/ml</td>
</tr>
<tr>
<td>Clone:</td>
<td>SK3 (also known as Leu3a)</td>
</tr>
<tr>
<td>Storage Buffer:</td>
<td>Aqueous buffered solution containing ≤0.09% sodium azide.</td>
</tr>
</tbody>
</table>

Description

The Human CD4 Fluorochrome Evaluation Kit includes 10µg of the following components:

- Alexa Fluor® 700 Mouse Anti-Human CD4 Evaluation Sample
- APC Mouse Anti-Human CD4 Evaluation Sample
- APC-Cy™7 Mouse Anti-Human CD4 Evaluation Sample
- APC-R700 Mouse Anti-Human CD4 Evaluation Sample
- BB515 Mouse Anti-Human CD4 Evaluation Sample
- BB700 Mouse Anti-Human CD4 Evaluation Sample
- BUVC95 Mouse Anti-Human CD4 Evaluation Sample
- BV496 Mouse Anti-Human CD4 Evaluation Sample
- BUVC63 Mouse Anti-Human CD4 Evaluation Sample
- BUVC661 Mouse Anti-Human CD4 Evaluation Sample
- BUVC737 Mouse Anti-Human CD4 Evaluation Sample
- BUVC805 Mouse Anti-Human CD4 Evaluation Sample
- BV421 Mouse Anti-Human CD4 Evaluation Sample
- BUVC615 Mouse Anti-Human CD4 Evaluation Sample
- BV421 Mouse Anti-Human CD4 Evaluation Sample
- BV480 Mouse Anti-Human CD4 Evaluation Sample
- BV510 Mouse Anti-Human CD4 Evaluation Sample
- BV605 Mouse Anti-Human CD4 Evaluation Sample
- BV650 Mouse Anti-Human CD4 Evaluation Sample
- BV711 Mouse Anti-Human CD4 Evaluation Sample
- BV750 Mouse Anti-Human CD4 Evaluation Sample
- BV786 Mouse Anti-Human CD4 Evaluation Sample
- PE Mouse Anti-Human CD4 Evaluation Sample
- PE-Cy™5 Mouse Anti-Human CD4 Evaluation Sample
- PE-Cy™7 Mouse Anti-Human CD4 Evaluation Sample
- PE-CF594 Mouse Anti-Human CD4 Evaluation Sample
- PerCP-Cy™5.5 Mouse Anti-Human CD4 Evaluation Sample
- FITC Mouse Anti-Human CD4 Evaluation Sample
- PE-CF594 Mouse Anti-Human CD4 Evaluation Sample
- PerCP-Cy™5.5 Mouse Anti-Human CD4 Evaluation Sample
- BV421 Mouse Anti-Human CD4 Evaluation Sample
- FITC Mouse Anti-Human CD4 Evaluation Sample

To access the TDS for individual conjugates, search the name of the clone and/or the specificity on the website:

http://www.bdbiosciences.com

Fluorochrome brightness is representative of the specific anti-Human CD4 clones, other clones may differ. As for all tandem reagents, spillover values should not be assumed to be identical for other clones.

Please note that the following dyes are detected in the same channel. To access theAbsorption and Emission Spectra for each individual fluorochrome, go to: http://www.bdbiosciences.com/us/applications/s/spectrumguidepage

- FITC, BB515
- BV510, BV480
- BB700, PerCP-Cy5.5
- Alexa Fluor® 700, APC-R700

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 under optimal conditions that minimize unconjugated dye and antibody.

Application Notes

Application

| Flow cytometry | Routinely Tested |

Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to BD CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and BD CompBead to ensure that BD CompBeads are appropriate for your specific cellular application.
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) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Note: When using high concentrations of antibody, background binding of this dye to erythroid cell subsets (mature erythrocytes and precursors) has been observed. For researchers studying these cell populations, or in cases where light scatter gating does not adequately exclude these cells from the analysis, this background may be an important factor to consider when selecting reagents for panel(s).

### Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<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>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>566644</td>
<td>Mouse CD4 Fluorochrome Evaluation Kit</td>
<td>1 Each</td>
<td>RM4-5, GK1.5</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. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
2. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. CF™ is a trademark of Biotium, Inc.
10. This product is provided under an Agreement between BIOTIUM and BD Biosciences. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications owned or licensed by Biotium, Inc. This product, and only in the amount purchased by buyer, may be used solely for buyer’s own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. This product is for research use only. Diagnostic uses require a separate license from Biotium, Inc. For information on purchasing a license to this product including for purposes other than research, contact Biotium, Inc., 3159 Corporate Place, Hayward, CA 94545, Tel: (510) 265-1027. Fax: (510) 265-1352. Email: btinfo@biotium.com.

11. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer’s own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.

12. Cy is a trademark of GE Healthcare.

13. The BD Horizon Brilliant™ Violet, Blue and Ultraviolet dyes and BD Horizon Brilliant Stain Buffer are covered by one or more of the following US patents: 8,110,673; 8,354,239; 8,158,444; 8,227,187; 8,362,193; 8,455,613; 8,575,303; 8,802,450.

14. 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.

15. BD Horizon Brilliant Violet 615 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.


17. BD Horizon Brilliant Ultraviolet 395 is covered by one or more of the following US patents: 8,158,444; 8,575,303; 8,354,239.

18. BD Horizon Brilliant Ultraviolet 496 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.

19. BD Horizon Brilliant Ultraviolet 563 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.

20. BD Horizon Brilliant Ultraviolet 661 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.

21. BD Horizon Brilliant Ultraviolet 737 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.

22. BD Horizon Brilliant Ultraviolet 805 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,575,303; 8,354,239.

23. 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.

24. BD Horizon Brilliant Violet 480 is covered by one or more of the following US patents: 8,575,303; 8,354,239.

25. BD Horizon Brilliant Violet 510 is covered by one or more of the following US patents: 8,575,303; 8,354,239.

26. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.

27. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from BD Horizon™ BV605 conjugate may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon™ BV605 conjugate.

28. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.

29. BD Horizon Brilliant Violet 650 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,455,613; 8,575,303; 8,354,239.

30. BD Horizon Brilliant Violet 711 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,455,613; 8,575,303; 8,354,239.

31. BD Horizon Brilliant™ Violet 750 is covered by one or more of the following US patents: 8,158,444; 8,802,450; 8,575,303; 8,455,613; 8,227,187; 8,841,072; 8,110,673.

32. BD Horizon Brilliant Violet 786 is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,227,187; 8,455,613; 8,575,303; 8,354,239.

33. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.

34. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.

35. BD Horizon Brilliant Blue 700 is covered by one or more of the following US patents: 8,455,613 and 8,575,303.

36. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.

37. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.

38. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.

94545, Tel: (510) 265-1027.  Fax: (510) 265-1352. Email: btinfo@biotium.com.
39. PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells. However, PE-Cy5 conjugated reagents should not be used to stain splenocytes of SJL, NOD, and MRL mice as B lymphocytes and/or other leukocytes have been reported to non-specifically stain regardless of the use of Mouse BD Fc Block™ (the CD72c complex has been implicated for PE-Cy5 binding in these strains). Reagents conjugated to PE, PerCP, PerCP-Cy5.5, APC, and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.

40. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).

41. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.

42. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.

43. This product is provided under an intellectual property license between Life Technologies Corporation and BD Businesses. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For information on purchasing a license to this product for any other use, contact Life Technologies Corporation, Cell Analysis Business Unit Business Development, 29851 Willow Creek Road, Eugene, OR 97402, USA, Tel: (541) 465-8300. Fax: (541) 335-0504.

44. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.

45. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.