1. INTENDED USE
BD Oncomark™ CD4 FITC/CD25 PE/CD3 PerCP-Cy™5.5® is intended for in vitro flow cytometric immunophenotyping of CD25 in normal and abnormal T cells.1-3 CD3+ T cells express the CD25 antigen after stimulation.4-7 T cells transformed by the human T-cell lymphotrophic virus also over-express CD25.6,7 Aberrant ratios of CD3+CD4+ and CD3+CD4− populations might develop in many T-cell disorders. CD4/CD25/CD3 assays are used in the diagnosis of hematologic disorders.1-3

2. COMPOSITION
CD4, clone SK3, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes.

CD25, clone 2A3, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with PHA-activated human T lymphocytes.

CD3, clone SK7,8-11 is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

CD4, CD25, and CD3 are each composed of mouse IgG1 heavy chains and kappa light chains.

* Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for in vitro diagnostics. It is not licensed for any other use. If you require any additional license to use this product and do not have one, return this material, unopened, to BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, and any money paid for the material will be refunded.
This reagent is supplied as a combination of CD4 FITC, CD25 PE, and CD3 PerCP-Cy5.5 in 1 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and 0.1% sodium azide.

Antibody purity is as follows.
- FITC, PE, PerCP-Cy5.5: ≤20% free fluorophore at bottling, as measured by size-exclusion chromatography (SEC)

3. STORAGE AND HANDLING
The antibody reagent is stable until the expiration date shown on the label when stored at 2°C–8°C. Do not use after the expiration date. Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the outside of the reagent vial dry.

Do not use the reagent if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.

4. REAGENTS OR MATERIALS REQUIRED BUT NOT PROVIDED
- Falcon® disposable 12 x 75-mm polystyrene test tubes or equivalent
- Micropipettor with tips
- Vortex mixer
- BD FACSTM lysing solution (10X) (Catalog No. 349202). For dilution instructions and warnings, refer to the instructions for use (IFU).
- Centrifuge
- BD CellWASH™ (Catalog No. 349524) or a wash buffer of PBS with 0.1% sodium azide
- BD CellFIX™ (Catalog No. 340181) or 1% paraformaldehyde solution in PBS with 0.1% sodium azide. Store at 2°C–8°C in amber glass for up to 1 week.

Properly equipped cytometer. Flow cytometers must have laser excitation set at 488 nm and must be equipped to detect light scatter and the appropriate fluorescence, and have the appropriate analysis software installed for data acquisition and analysis. Refer to your instrument user's guide for instructions.

5. SPECIMEN(S)
BD Oncomark CD4 FITC/CD25 PE/CD3 PerCP-Cy5.5 can be used for immunophenotyping by flow cytometry with peripheral blood and bone marrow aspirates collected in BD Vacutainer® EDTA tubes. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.12,13

WARNING
All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection14,15 and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

6. PROCEDURE
Viability of samples should be assessed and a cutoff value established. A cutoff value of at least 80% viable cells has been suggested.12
To avoid serum interference when using these reagents, it is necessary to pre-wash the sample using at least 25 volumes excess 1X PBS with 0.1% sodium azide (48 mL of 1X PBS with sodium azide per 2 mL of whole blood to be washed). Mix well. Pellet cells by centrifugation and resuspend in 1X PBS with 0.1% sodium azide to the original volume.

1. Add 20 µL of BD Oncomark CD4/CD25/CD3 reagent to 100 µL of whole blood or prefiltered bone marrow in a 12 x 75-mm tube.
2. Vortex gently and incubate for 15 to 20 minutes in the dark at room temperature (20°C–25°C).
3. Add 2 mL of 1X BD FACS lysing solution.
4. Vortex gently and incubate for 10 minutes in the dark at room temperature.
5. Centrifuge at 300g for 5 minutes. Remove the supernatant.
6. Add 2 to 3 mL of BD CellWASH solution (or wash buffer) and centrifuge at 200g for 5 minutes. Remove the supernatant.
7. Add 0.5 mL of BD CellFIX solution or 1% paraformaldehyde and mix thoroughly. Store at 2°C–8°C until analyzed.

Stained samples should be analyzed within 24 hours of staining.

**Flow Cytometric Analysis**

1. Set up the instrument as recommended by the manufacturer.
   Run a control sample daily from a normal adult subject or a commercially available whole blood control to optimize instrument settings and as a quality control check of the system.
2. Vortex the cells thoroughly at low speed to reduce aggregation before running them on the flow cytometer.16
3. Run the sample on the flow cytometer. Verify that all populations are on scale. Optimize the instrument settings, if needed.
4. Acquire and analyze list-mode data using appropriate software.
5. On the appropriate plots, use the required combination of gates, regions, or quadrants to isolate the population of interest (Figure 1).

![Figure 1](#)  
**Figure 1** Dot plots displaying region R1 and quadrants

6. Determine antigen expression based on the sample negative population.
7. PERFORMANCE CHARACTERISTICS

Specificity

The CD4\textsuperscript{17,18} antigen, with a molecular weight of 55 kilodalton (kDa),\textsuperscript{8} is present on T-helper/inducer lymphocytes and monocytes.\textsuperscript{19,20}

CD25 recognizes an antigen that is the human low-affinity interleukin-2 receptor (IL-2R).\textsuperscript{21,22} The antigen has a molecular weight of 55 kDa.\textsuperscript{23}

CD3 reacts with the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.\textsuperscript{24} The antigen recognized by CD3 antibodies is noncovalently associated with either \(\alpha/\beta\) or \(\gamma/\delta\) TCR (70 to 90 kDa).\textsuperscript{25}

Antigen Distribution

The CD4 antigen is present on the helper/inducer T-lymphocyte subset (CD3\textsuperscript{+}CD4\textsuperscript{+}) that comprises 28% to 58%\textsuperscript{26} of normal peripheral blood lymphocytes.\textsuperscript{8,18} The CD4 antigen is present in low density on the cell surface of monocytes.

The CD25 (Anti–IL-2R) antigen is present on a subset of peripheral blood lymphocytes.\textsuperscript{27}

The CD3 antigen is present on 61% to 85% of normal peripheral blood lymphocytes.\textsuperscript{26}

8. LIMITATIONS

Use of this reagent combination for diagnostic evaluation of hematologic disorders should be performed in the context of a thorough immunophenotypic analysis including other relevant markers.

Procedures using BD Oncomark reagents must adhere to the instructions for use for the specific instrument, software, and quality control procedures used by your laboratory.

Reagent performance data was collected typically with EDTA-treated specimens. Reagent performance can be affected by the use of other anticoagulants.

Samples with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD’S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.
### TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor resolution between debris and lymphocytes</td>
<td>Cell interaction with other cells and platelets</td>
<td>Prepare and stain another sample.</td>
</tr>
<tr>
<td>Rough handling of cell preparation</td>
<td>Check cell viability; centrifuge cells at lower speed.</td>
<td></td>
</tr>
<tr>
<td>Inappropriate instrument settings</td>
<td>Follow proper instrument setup procedures; optimize instrument settings as required.</td>
<td></td>
</tr>
</tbody>
</table>

**Staining dim or fading**

| Cell concentration too high at staining step | Check and adjust cell concentration or sample volume; stain with fresh sample. |
| Insufficient reagent | Repeat staining with increased amount of antibody. |
| Cells not analyzed within 24 hours of staining | Repeat staining with fresh sample; analyze promptly. |

**Few or no cells**

| Cell concentration too low | Resuspend fresh sample at a higher concentration; repeat staining and analysis. |
| Cytometer malfunctioning | Troubleshoot instrument. |

### REFERENCES


