**EVIDENCE OF DETERIORATION**

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following email address: immuno-techsup@beckmancoulter.com.

**PRECAUTIONS**

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Let it come to room temperature (18 – 25°C) before use.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide (NaN₃) should be handled with care. Do not take internally and avoid all contact with the skin, mucosa and eyes.

**SAMPLES**

Venous blood must be taken using sterile tubes containing an EDTA salt as the anti-coagulant. The samples should be kept at room temperature (18 – 25°C) and not shaken. The samples should be homogenized by gentle agitation prior to taking the test sample. The samples must be analyzed within 24 hours of venipuncture.

**METHODOLOGY**

**NECESSARY MATERIAL NOT SUPPLIED**

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposal tips for 10, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 66070607).
- Red cell lysis reagent with washing stage after lysis. For example: VersaLyse™ (Ref. A09777).

Leucocyte fixation reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).


Buffer: PBS (0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).

Centrifuge.

Automatic agitator (Vortex type).

Flow cytometer.

**PROCEDURE**

**NOTE:** The procedure below is valid for standard applications. Sample and/or VersaLyse volumes for certain Beckman Coulter applications may be different. If such is the case, follow the instructions on the application’s technical leaflet.

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed in the presence of the isotypic control (Ref. A07798).

1. Add 10 µL of specific IOTest conjugated antibody to each test tube, and 10 µL of the isotypic control to each control tube.
2. Add 100 µL of the test sample to both tubes. Vortex the tubes gently.
3. Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4. Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used. As an example, if you wish to use VersaLyse (Ref. A09777), refer to the leaflet and follow preferably the procedure called “with concomitant fixation”, which consists of adding 1 mL of the “Fix-and-Lyse™” mixture prepared extemporaneously. Vortex immediately for one second and incubate for 10 minutes at room temperature, protected from light.
5. If the sample does not contain red cells, add 2 mL of PBS.
6. Centrifuge for 5 minutes at 150 x g at room temperature.
7. Remove the supernatant by aspiration and resuspend the cell pellet using 3 mL of PBS.
8. Repeat step 5.
9. Remove the supernatant by aspiration and resuspend the cell pellet using:
   - 0.5 mL or 1 mL of PBS plus 0.1% of formaldehyde if the preparations are to be kept for more than 2 hours and less than 24 hours. (A 0.1% formaldehyde PBS can be obtained by diluting 12.5 µL of the IOTest 3 Fixative Solution (Ref. A07800) at its 10X concentration in 1 mL of PBS).
   - 0.5 mL or 1 mL of PBS without formaldehyde, if the preparations are to be analyzed within 2 hours.
PERFORMANCE
Performance data are obtained using the procedure described above on 24 hour-old blood samples previously collected on sterile tubes with EDTA salt as anticoagulant. Analysis was performed within 2 hours following immunostaining.

SPECIFICITY
The CD117 antigen, also known as Stem Cell Factor Receptor (SCFR), mast-cell-Kit, and steel factor receptor, is a 145 kDa transmembrane glycoprotein encoded by the c-kit proto-oncogen (1).

The CD117 molecule belongs to the class III Receptor Tyrosine Kinase (RTK) family. Within the hematopoietic compartment, the CD117 molecule is expressed on approximately 50% of CD34+ progenitors engaged in erythropoietic differentiation (7), myelo-monocytic and megakaryocytic differentiation (2, 8).

Although CD117 is primarily a marker for nonlymphoid progenitor, it has been reported to be detected on early lymphoid progenitor (8, 9). CD117 expression has been found on a small subset of resting NK cells (CD56brightCD117), and about 30% of immature CD3– CD4– CD8– thymocytes (2). CD117 is also expressed on mast cells (2, 8) and detected on nonhematopoietic cells such as reproductive system, melanocytes and embryonic brain (8).

MAB 104D2D1 was assigned to CD117 during the 6th HLDA Workshop on Human Leucocyte Differentiation Antigens held in Kobe, Japan, in 1996 (WS Code: C-30. Section C) (8).

LINEARITY
To test the linearity of staining of this reagent, a positive cell line (MO7E) and a negative cell line (RPMI8866) were mixed in different proportions with a constant final number of cells, so that the mixture ranged from 0 to 100%.

Aliquots were stained using the procedure described above and linear regression between the expected values and the observed values was calculated.

EXPECTED VALUES
Each laboratory must compile a list of reference values based upon a group of healthy donors from the local population. This must be done by taking age, sex and ethnic group into account, as well as any other potential regional differences.

In our laboratories, the whole blood samples of 10 healthy adults were treated using the reagent described above. The results obtained for the count of the positive events of interest with this reagent are given in the tables below:

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD117 *</td>
<td>10</td>
<td>2.21</td>
<td>0.79</td>
<td>35.9</td>
</tr>
</tbody>
</table>

INTRA-LABORATORY REPRODUCIBILITY
On the same day and using the same cytometer, 12 measurements of the percentage of staining of a positive target were carried out. The results obtained are summarized in the following table:

<table>
<thead>
<tr>
<th>Positive Target</th>
<th>Number</th>
<th>Mean (%)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD117 *</td>
<td>12</td>
<td>37.87</td>
<td>0.57</td>
<td>1.49</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE TECHNIQUE
1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence leaks have not been correctly compensated for and if the regions have not been carefully positioned.

2. It is preferable to use a RBC lysis technique with a washing step as this reagent has not been optimized for "no wash" lysis techniques.

3. Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert leaflet and compatible with good laboratory practices.

4. The conjugated antibody of this reagent is calibrated so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test.

5. In the case of a hyperleucocytosis, dilute the blood in PBS so as to obtain a value of approximately 5 x 10⁹ leucocytes/L.

6. In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is recommended to isolate mononucleated cells using a density gradient (Ficoll, i.e. for example) prior to staining.

7. Due to the tandem structure of the fluorochrome, PC5 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC5. Therefore, for multicolor analysis, the compensation matrix should be carefully checked when changing the lot of a PC5-conjugate.

NOTE: In all cases, keep the preparations between 2 and 8°C and protected from light.
The graph below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lyzed normal whole blood sample. Staining is with IOTest CD117-PC5 Conjugated Antibody (Ref. IM2733). All leucocytes are represented. Analysis is performed with a CYTOMICS FC 500 flow cytometer equipped with CXP Analysis Software.

REFERENCES