Instruments

NAVIOS, Beckman Coulter
KALUZA analysis software, Beckman Coulter
Centrifuge
Vortexer
Refrigerator
Pipettes
Pipette boy

Materials

Pipette tips (5 μl - 1000 μl) for low volume (μl) pipettes
Pipette tips for pipette boy
12x75mm-Flow cytometry tubes
Rack for 12x75mm-tubes

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Order no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IsoFlow™</td>
<td>Beckman Coulter</td>
<td>8546859</td>
</tr>
<tr>
<td>Versa Lyse™ – Lysing solution</td>
<td>Beckman Coulter</td>
<td>A09777</td>
</tr>
<tr>
<td>IOTest® 3 Fixative solution (10x)</td>
<td>Beckman Coulter</td>
<td>A07800</td>
</tr>
<tr>
<td>Test tube, 12 x 75 mm Blue, 250 pieces</td>
<td>Beckman Coulter</td>
<td>2523749</td>
</tr>
<tr>
<td>FCS – head inactivated or an other comparable commercial product</td>
<td>Biochrom</td>
<td>S0115</td>
</tr>
<tr>
<td>NaN or an other comparable commercial product</td>
<td>Merck</td>
<td>1.06549.0100</td>
</tr>
</tbody>
</table>

Working solutions / buffer

<table>
<thead>
<tr>
<th>PBS/FCS-buffer</th>
<th>IsoFlow + 0,1% NaN3 + 2 % FCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x Versa Lyse-Fix-solution</td>
<td>10 ml Versa-Lyse + 250 μl IOTest 3 Fixative solution (10x)</td>
</tr>
</tbody>
</table>

Antibodies and pipetting volumes

<table>
<thead>
<tr>
<th>Antibody Panel</th>
<th>Company</th>
<th>Volume per test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM01</td>
<td>Beckman Coulter</td>
<td>40 μl (20μl vial1 + 10μl vial2 + 10μl vial3)</td>
</tr>
<tr>
<td>BM02</td>
<td>Beckman Coulter</td>
<td>40 μl (20μl vial1 + 10μl vial2 + 10μl vial3)</td>
</tr>
<tr>
<td>BM03</td>
<td>Beckman Coulter</td>
<td>40 μl (20μl vial1 + 10μl vial2 + 10μl vial3)</td>
</tr>
<tr>
<td>BM04</td>
<td>Beckman Coulter</td>
<td>40 μl (20μl vial1 + 10μl vial2 + 10μl vial3)</td>
</tr>
<tr>
<td>BM06</td>
<td>Beckman Coulter</td>
<td>40 μl (20μl vial1 + 10μl vial2 + 10μl vial3)</td>
</tr>
</tbody>
</table>

Material for analysis

<table>
<thead>
<tr>
<th>Material</th>
<th>EDTA whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storing conditions</td>
<td>transport at room temperature, storing in the laboratory at 4 °C</td>
</tr>
<tr>
<td>Storage times</td>
<td>max. 4h after blood collection, max 12h after end of staining</td>
</tr>
</tbody>
</table>
Sample preparation

01. EDTA-whole blood store within refrigerator, mix well prior to use
02. Label four 5ml flow cytometry tubes with „ONE 01 to ONE 04“ and patient ID
03. Label two 5ml flow cytometry tubes with “ONE 06” and patient ID
04. Add 100 µl EDTA-blood per tube

Surface staining

05. Add antibody mixes according to label on tube
06. Vortex for 10 s
07. Incubate for 15 min, RT (room temperature), in the dark
08. Vortex for 10 s
09. Add 1,5 ml 1x Versa-Lyse-Fix-solution to all tubes
10. Vortex briefly
11. Incubate for 15 min, RT (room temperature), in the dark
12. Vortex for 5 s
13. Add 2,5 ml cold PBS/FCS-buffer to all tubes
14. Centrifuge, 300 g, 5 min, 4 °C
15. Decant
16. Vortex for 5 s
17. Add cell suspension from one tube “ONE 06” to the second tube “ONE 06”
18. Add 3 ml PBS/FCS to all tubes
19. Centrifuge, 300 g, 5 min, 4 °C
20. Decant
21. Vortex for 5 s
22. Add 150 µl PBS/FCS-buffer (resulting in final volume about 250 µl)

Store at 4 °C in the dark until measurement

Setting information for NAVIOS cytometer

Protocol:  
BM01 (for the stained blood sample tube BM01)  
BM02 (for the stained blood sample tube BM02)  
BM03 (for the stained blood sample tube BM03)  
BM04 (for the stained blood sample tube BM04)  
BM05 (for the stained blood sample tube BM05)

Cytosettings: ONE study Panel 01_2012 settings (for all tubes)

Sample designation:  
ID1: Donor ID  
ID2: Study  
ID3: Panel  
ID4: Analyst (three letter code: 1st letter “name” + 1st & 2nd letter “last name”)

Example: R5001V01_ONE-Study_BM01_MST

Supplemental Material

TDS VersaLyse Beckman Coulter  
TDS IOTest3 Fixative Solution Beckman Coulter  
TDS IsoFlow Beckman Coulter