Human BCR/ABL protein Kit

**Reference:** BCR-ABL

**Size:** 25 test

**INTRODUCTION**

Immunobead assay for BCR/ABL human fusion protein detection. The Philadelphia chromosome or Philadelphia translocation is a specific genetic abnormality in chromosome 22 of leukemia cancer cells (particularly chronic myelogenous leukemia (CML) cells). This chromosome is defective and unusually short because of reciprocal translocation of genetic material between chromosome 9 and chromosome 22, and contains a fusion gene called BCR-ABL. This gene is the ABL gene of chromosome 9 juxtaposed onto the BCR gene of chromosome 22, coding for a hybrid protein: a tyrosine kinase signalling protein that is "always on", causing the cell to divide uncontrollably. BCR–ABL fusion proteins show increased signaling through their ABL tyrosine kinase domain, which can be blocked by specific inhibitors, thereby providing effective treatment. This makes detection of BCR–ABL aberrations of utmost importance for diagnosis, classification and treatment of leukemia patients.

**PRODUCT DESCRIPTION**

The kit is a simple flow cytometric immunobead assay for detection of BCR–ABL fusion proteins in cell lysates, using a bead-bound anti-BCR capture antibody and a fluorochrome conjugated anti-ABL detection antibody (Fig. 1). The kit provides results within 4 h, and can be run in parallel to routine immunophenotyping.

Tested application: Flow Cytometry

Species reactivity: Human

Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN₃).

Recommended usage: Immunostep BCR-ABL protein kit, is intended for determining BCR-ABL fusion proteins by Flow Cytometry in cell lysates.

Presentation: liquid/lyophilized

![Image of protein complex formation](image)

Figure 1: Formation of sandwich complex in the BCR-ABL immunoassay.

**REAGENTS PROVIDED**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>COMPONENTS</th>
<th>AMOUNT</th>
<th>Nº TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment Buffer</td>
<td>Lyophilized PBS Na₂HPO₄ + Protease inhibitors cocktail</td>
<td>500 µl/test</td>
<td>25</td>
</tr>
<tr>
<td>Lysis Buffer</td>
<td>Lyophilized Lysis Solution + Protease inhibitors cocktail</td>
<td>150 µl/test</td>
<td>25</td>
</tr>
<tr>
<td>Positive control</td>
<td>K562 lyophilized cell lysate (positive control)</td>
<td>5 million/test</td>
<td>2</td>
</tr>
<tr>
<td>Magnetic Capture Beads</td>
<td>Anti-human BCR (Clone 3E2C10) capture beads, Polystyrene micro-particles with Mean Diameter (µm) 6,5±0,2 (CV±5%), having discrete fluorescence intensity characteristics</td>
<td>100 µl/test</td>
<td>25</td>
</tr>
<tr>
<td>Detector Antibody</td>
<td>Anti-human ABL-Phycoerythrin (PE) (Clone 8E9). The excitation of PE by 488 nm laser light induces a light emission maximum of 575 nm.</td>
<td>50 µl/test</td>
<td>25</td>
</tr>
<tr>
<td>Assay Buffer 10X</td>
<td>PBS BBR-5%. Do not freeze. Dilute to 1X for use in this assay.</td>
<td>20 ml</td>
<td></td>
</tr>
</tbody>
</table>

**APPROPRIATE STORAGE AND HANDLING CONDITIONS**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>PREPARATION</th>
<th>STATE</th>
<th>STORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment Buffer</td>
<td>Reconstitute with 6.25 ml of demineralized water. Allow to stabilize in liquid for at least 5 minutes</td>
<td>Lyophilized</td>
<td>-2° to -8°C (preferably aliquoted)</td>
</tr>
<tr>
<td>Lysis Buffer</td>
<td>Reconstitute with 3.75 ml of demineralized water.</td>
<td>Lyophilized</td>
<td>-2° to -8°C (preferably aliquoted)</td>
</tr>
<tr>
<td>Positive control</td>
<td>Reconstitute with (100 µl of demineralized water.</td>
<td>Lyophilized</td>
<td>-2° to -8°C (preferably aliquoted)</td>
</tr>
<tr>
<td>Magnetic Capture Beads</td>
<td>Ready to use</td>
<td>Liquid</td>
<td>-2° to -8°C</td>
</tr>
<tr>
<td>Detector Antibody</td>
<td>Ready to use</td>
<td>Liquid</td>
<td>-2° to -8°C</td>
</tr>
<tr>
<td>Assay Buffer 10X</td>
<td>Dilute to 1X for use in this assay.</td>
<td>Liquid</td>
<td>-2° to -8°C</td>
</tr>
</tbody>
</table>

Store in the dark, refrigerated between 2 °C and 8 °C. The kit is stable until the expiry date stated on the vial label if kept at 2-8°C. Check lysis and pretreatment buffers storage conditions once suspended. Do not use after the date indicated.

**REAGENTS NOT PROVIDED.**

- Magnetic Rack; MagNeSphere(R) Mag. Sep. Stand 12-hole, 12x75mm (PRoMega, Ref Z5343).
- 12x75 mm Polystyrene Round Bottom Tubes (cytometer tubes).

**EVIDENCE OF DETERIORATION**

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

**RECOMMENDATIONS AND WARNINGS**

a) Avoid microbial contamination of the reagent. Assay buffer 1X can be filtered before use.

b) Microspheres and reagents should be protected from prolonged exposure to light throughout this procedure.

c) The samples should be treated with appropriate handling procedures.

d) Do not use after the expiry date indicated on the vial.

e) Deviations from the recommended procedure could invalidate the analysis results.

f) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.
The detection success is dependent on the quality of cell lysis process and the time elapsed from the sample collection. In this sense sample must be processed as soon as possible, with a maximum time limit of 2 hours from the time of collection.

For professional use only.

**WARRANTY**

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep’s sole liability is limited to either the replacement of the products or refund of the purchase price.

**SAMPLE PREPARATION PROTOCOL**

Blood samples from patients suspected to have CML were exclusively processed by NH₄Cl lysis of the erythrocytes (not by ficoll separation) to avoid selective loss of the CML cells. In case of ALL and AML samples, either NH₄Cl lysis or ficoll separation were used for cell processing.

Once the appropriate cells are separated, process it as follows:

a) Protease Inhibitor pretreatment. To inhibit proteases before the cells are lysed, intact cells are pretreated with reconstituted Pretreatment Buffer. Resuspend pelleted cells in 0.5ml pretreatment buffer (maximum 5x10⁶cells/ml) and incubate for 10 min on ice. Centrifuge cells (5 min at 540 xg at 4 ºC), remove the supernatant completely and wash cells immediately, as described below.

b) Wash cells with 1ml of 5% fetal bovine serum in PBS 1X. Centrifuge cells (5 min at 540 xg at 4 ºC), remove the supernatant completely and lyse the cells immediately, as described below.

c) After removal of the supernatant, cell lysates are prepared by resuspending the pelleted cells in reconstituted Cell Lysis Buffer (5 million cells/150 µl of buffer). After 30min on ice, centrifugate the lysate 17000 xg at 4ºC 10min (recommended) or room temperature for 2 min to remove cell debris. Use the supernatant in a cytometric bead assay. (Annex 1)

5. After incubation period, add 50 µL of the Detection antibody solution to each tube. Mix the reactions gently by pipetting up and down several times with a pipettor.

6. Incubate for 60 minutes at RT in an orbital shaker. Protect it from light.

7. Add 0.5ml of PBS 1X and wash the microspheres by gently vortexing for approximately 5 seconds.

8. Collect the Magnetic beads by placing tubes on a magnetic rack and incubate 5 minutes or by centrifugation at 2500xg for 5min. Remove supernatant from tubes by hand-decanting (Fig. 4) or by aspiration. Take care not to disturb the microspheres, and make sure not to leave more than 100 ul of supernatant in the tube.

9. Remove the tubes from the magnetic rack and resuspend the microspheres in 200 µL of PBS 1X by gently vortexing for approximately 5 seconds. (Annex 2)

PROTEIN ASSAY PROTOCOL.

1. Resuspend the Working Microspheres Solution by vortex for approximately 20 seconds.

2. Prepare one 12x75mm Round Bottom Polystyrene for each sample to analyze. Add 100 µL of the Working Microsphere Solution to each 12x75 mm Polystyrene Round Bottom tube.

3. Add the sample previously prepared according to “Sample Preparation Protocol” to the appropriate tubes. Mix the reactions gently by pipetting up and down several times with a pipettor.

4. Incubate for 120 minutes at room temperature (RT) in an orbital shaker. Protect it from light.

10. Acquire in the cytometer as indicated below.
ASSAY ACQUISITION

An adequate gating strategy FSC / SSC and PerCP/APC, helps to bead population identification and discrimination of doublets on flow cytometer.

1. Gate on the single population(s) on a Forward Scatter vs. Side Scatter plot in linear scale. (Figure 5, A)
2. Gate on the single population(s) on a PerCP/APC channel (bead auto fluorescence) in logarithmic scale (Figure 5, B)
3. Using the PE parameters to determine whether or not any bead populations tested “positive” for the BCR/ABL fusion protein. Note: A positive bead will produce a fluorescent peak in the PE channel.

FLOW CYTOMETRY ANALYSIS

The bead assay provide a way to capture the soluble analyte (BCR/ABL fusion protein) making possible to detect BCR/ABL fusion proteins in human blood research samples

Figure 3: Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and PerCP vs APC (B)

Figure 4: Flow analysis of BCR/ABL fusion protein. Once cell lysates are prepared using BCR/ABL Protein Kit and samples are run in the flow cytometer, dot-plots (left) and histograms (right) show the presence (top) or absence (bottom) of BCR/ABL fusion proteins.

REFERENCES


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Annex 1: Sample preparation workflow for fusion protein detection.

Annex 2: Sample preparation workflow for fusion protein detection.