Human BCR/ABL protein Kit

REF	Σ
BCR-ABL	25 test

1. 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.

2. PRODUCT DESCRIPTION

The kit is a robust flow cytometric immunobead assay designed for the accurate detection of BCR-ABL fusion proteins in cell lysates. It uses a bead-bound anti-BCR antibody for protein capture and a fluorochrome-conjugated anti-ABL antibody for detection, ensuring high specificity and sensitivity (Fig. 1).

The assay is optimised to provide reliable results within 4 hours and can be seamlessly integrated into standard immunophenotyping workflows, allowing efficient parallel processing.

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

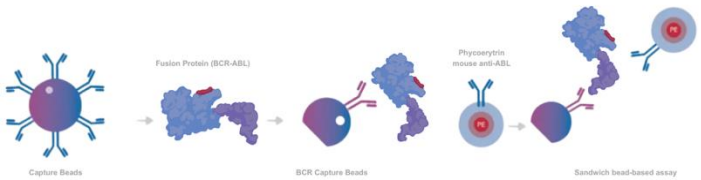

Figure 1: Formation of sandwich complex in the BCR-ABL immunoassay.

3. REAGENTS PROVIDED

DESCRIPTION	COMPONENTS	AMOUNT	Nº TEST
Pretreatment Buffer 10X	Lyophilized PBS Na^+/K^+ + Protease inhibitors cocktail	1 ml/ test (1X)	25
Cell Lysis Buffer 1X	Lyophilized Lysis Solution + Protease inhibitors cocktail	50 µl/test	25
BCR- Capture Beads	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	10 µl/test	25
Detector Antibody (PE human anti-ABL)	Anti-human ABL-Phycoerythrin (PE) (Clone 8E9). The excitation of PE by 488 nm laser light induces a light emission maximum of 575 nm	5 µl/test	25
Wash Buffer A 10X	PBS Na^+/K^+ BFS 50%. Dilute it to 1X in PBS NA^+/K^+	3 ml	
Wash Buffer B 10X	PBS 10% BSA, pH 7,4 – 10X. Do not freeze	6 ml	

4. APPROPRIATE STORAGE AND HANDLING CONDITIONS

DESCRIPTION	PREPARATION	STATE	STORE
Pretreatment Buffer 10X	Reconstitute the reagent using 2.5 mL of demineralized water. Allow it to stabilize in liquid form for a minimum of 5 minutes to ensure complete dissolution. Subsequently, dilute the solution from a 10X concentration to a 1X working concentration using PBS containing Na^+/K^+.	Lyophilized	2-8ºC
		Liquid (reconstituted)	-20ºC (preferably aliquoted)
Lysis Buffer 1x	Reconstitute with 1.25 ml of demineralized water.	Lyophilized	2-8ºC
		Liquid (reconstituted)	-20ºC (preferably aliquoted)
Magnetic Capture Beads	Ready to use	Liquid	2-8ºC
Detector Antibody	Ready to use	Liquid	2-8ºC
Wash Buffer A 10X	Dilute the Wash Buffer A from its 10X concentrated solution to a 1X working solution using PBS containing Na^+/K^+. Ensure thorough mixing to achieve a homogeneous solution.	Liquid	2-8ºC
Wash Buffer B 10X	Dilute the Wash Buffer B from its 10X concentrated solution to a 1X working solution using PBS containing 1% BSA and Na^+/K^+. Ensure complete mixing for a uniform solution.	Liquid	2-8ºC

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

5. REAGENTS NOT PROVIDED

- Magnetic Rack; MagneSphere(R) Mag. Sep. Stand 12-hole, 12x75mm (PROMEGA, Ref Z5343).
- 12x75 mm Polystyrene Round Bottom Tubes (cytometer tubes).
- Positive control. Positive control. We recommend using the specified number of cells for the assay (1,25 million per test) from a cell line positive for the t(9;22) translocation or the BCR-ABL fusion gene, such as the lymphoblastic cell line K562, or from a sample previously identified as positive for t(9;22), provided it contains a percentage of pathological cells greater than 50%.
- Negative control. We recommend using the specified number of cells for the assay (1,25 million per test) from a cell line negative for the t(9;22) translocation or the BCR-ABL fusion gene, such as the MCF7 breast cancer cell line or a sample previously identified as negative for t(9;22).
- Ammonium chloride (NH_4Cl) lysis buffer.
- PBS Na + /K + . May be substituted with standard phosphate-buffered saline (PBS).

6. 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

7. RECOMMENDATIONS AND WARNINGS

1. Keep kit components away from direct light exposure during the protocol. Fluorescently conjugated antibodies and microspheres are sensitive to light.
2. Do not use after the expiration date specified on the vial label.. Reagents must not be used if the packaging shows clear evidence of deterioration.
3. Before starting the analysis, read the instructions carefully. Deviations from the recommended procedures may invalidate the assay results. Do not substitute or mix Immunostep kit reagents with reagents from other manufacturers..
4. Before acquiring samples, it is necessary to ensure that the flow cytometer settings and their compensation are appropriate.
5. 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.
6. During the procedure, specifically for the preparation of buffer dilutions included in the kit, PBS containing Na^+/K^+ may be substituted with standard phosphate-buffered saline (PBS), provided the former is not available.
7. For professional use only.

8. 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.

9. SAMPLE PREPARATION PROTOCOL

The kit is provided to assay $1{,}25x10^6$ cells per test.

Blood samples from patients suspected to have CML were exclusively processed by NH_4Cl lysis of the erythrocytes (not by ficoll separation) to avoid selective loss of the CML cells. It is recommended to lyse at a ratio of 1:25 (maximum of 2mL of blood to which 48mL of $NH4Cl$ lysis buffer is added). After 15 min of incubation in a sample-shaker device, centrifuge at 800 g for 10 min and remove the supernatant. In case of ALL and AML samples, either NH_4Cl lysis or ficoll separation were used for cell processing.

Once the appropriate cells have been separated, the cells are washed once with PBS Na^+/K^+ and processed as follows:

a) Protease Inhibitor pretreatment. To inhibit proteases before the cells are lysed, intact cells are pretreated with reconstituted Pretreatment Buffer (This buffer must be diluted from its 10X stock solution to a 1X working concentration using PBS containing Na^+/K^+ prior to use). Resuspend pelleted cells with 1 ml of Pretreatment Buffer 1X and incubate 10 min on ice. Afterwards, centrifuge cells (5 min at 540 xg at 4 ºC), remove the supernatant completely and wash cells immediately, as described below.Prepare the Wash buffer A. Dilute the 10X to 1X in PBS Na^+/K^+.

b) Wash cells with 1ml of Wash buffer A 1X (This buffer must be diluted its 10X stock solution to a 1X working concentration using PBS containing Na^+/K^+ prior to use). Afterwards, centrifuge cells (5 min at 540xg at 4 ºC), remove the supernatant completely and lyse the cells immediately, as described below.

c) After discarding the supernatant, prepare cell lysates by resuspending the pelleted cells in 50 µL of reconstituted Cell Lysis Buffer. Incubate the suspension on ice for 30 minutes, then centrifuge the lysate at 17,000 × g for 2 minutes at 4 °C. Use the resulting supernatant for the cytometric bead assay (refer to Annex 1).

10. PROTEIN ASSAY PROTOCOL

1. Prepare one 12x75mm Round Bottom Polystyrene for each sample to analyze.
2. The BCR-Capture Beads tend to precipitate; therefore, it is necessary to resuspend them by vortexing for approximately 20 seconds before dispensing. Immediately add 10 µL of the BCR-Capture Beads to each 12 × 75 mm polystyrene round-bottom tube that has been prepared in advance.
3. Add 50 µL of the previously prepared sample lysate, as outlined in the "Sample Preparation Protocol," to the polystyrene round-bottom tube containing the BCR-Capture Beads. Gently mix the reaction by vortexing for 5 seconds.
4. Incubate for 120 minutes at room temperature (RT) in an orbital shaker. Protect it from light.
5. After incubation, add 1ml of Wash buffer B 1X (This buffer must be diluted its 10X stock solution to a 1X working concentration using PBS containing Na^+/K^+ prior to use) and wash the BCR-capture Beads by gently vortexing for approximately 5 seconds.
6. Collect the BCR-Capture Beads (note that these beads are magnetic) by placing the tubes on a magnetic rack and incubating for 5 minutes. If a magnetic rack is unavailable, centrifuge the tubes at 2500 × g for 5 minutes. Remove the supernatant from the tubes either by hand-decanting (Fig. 2) when using a magnetic rack or by aspiration if centrifugation was used. Exercise caution to avoid disturbing the microspheres and ensure that no more than 100 µL of supernatant remains in the tube.
7. After removing the supernatant as described in the previous step, add 5 µL of the Detector Antibody to each 12 × 75 mm polystyrene round-bottom. Gently mix the reactions by pipetting up and down several times using a pipette.
8. Incubate for 60 minutes at RT in an orbital shaker. Protect it from light.
9. After incubation, add 1ml of Wash buffer B 1X (This buffer must be diluted its 10X stock solution to a 1X working concentration using PBS containing Na^+/K^+ prior to use) and wash the microspheres by gently vortexing for approximately 5 seconds.
10. Collect the sandwich complex (BCR-Capture Beads bound to the fusion protein and Detector Antibody) by placing the tubes on a magnetic rack and incubating for 5 minutes. If a magnetic rack is not available, centrifuge the tubes at 2500 × g for 5 minutes. Remove supernatant from tubes by hand-decanting (Fig. 2) or by aspiration. Remove the supernatant from the tubes either by hand-decanting (Fig. 2) when using a magnetic rack or by aspiration if centrifugation was used. Exercise caution to avoid disturbing the microspheres and ensure that no more than 100 µL of supernatant remains in the tuve
11. Remove the tubes from the magnetic rack and resuspend the microspheres in 200 µL of Wash buffer B 1X (This buffer must be diluted its 10X stock solution to a 1X working concentration using PBS containing Na^+/K^+ prior to use) by gently vortexing for approximately 5 seconds. (Annex 2).
12. Proceed to acquisition using the cytometer as outlined in the instructions below.

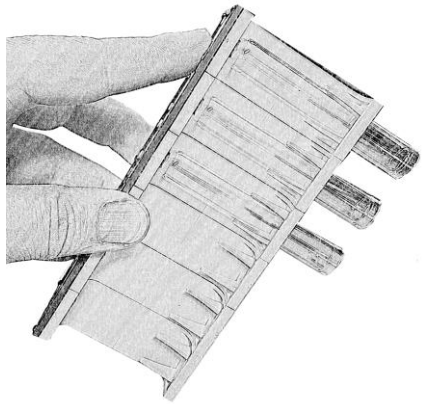

Figure 2: Hand-decanting supernatant using a Magnetic Rack.

11. 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 3)
2. Gate on the single population(s) on a PerCP/APC channel (bead auto fluorescence) in logarithmic scale (Figure 3)
3. Using the PE parameters (Figure 4) 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.

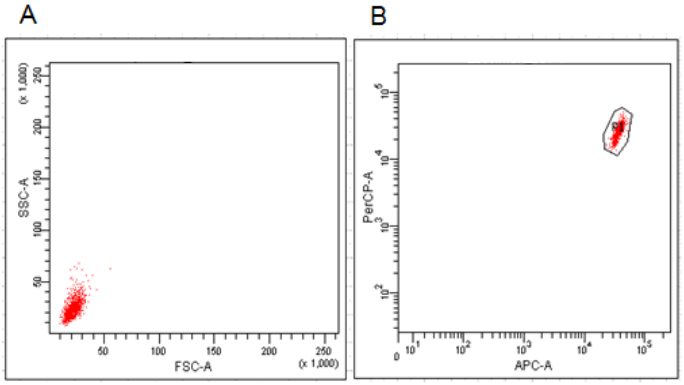

Figure. 3. Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and PerCP vs APC (B)

12. FLOW CYTOMETRY ANALYSIS

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

13. REFERENCES

1. F Weerkamp, E Dekking, YY Ng, VHJ van der Velden , H Wai , S Bo ¨ ttcher , M Bru ¨ ggemann , AJ van der Sluijs , A Koning , N Boeckx , N Van Poecke , P Lucio , A Mendonc¸a , L Sedek , T Szczepan´ ski , T Kalina , M Kovac , PG Hoogeveen , J Flores-Montero, A Orfao, E Macintyre, L Lhermitte, R Chen , KAJ Brouwer-De Cock, A van der Linden, AL Noordijk, WM Comans-Bitter, FJT Staal1 and JJM van Dongen1 On behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). Flow cytometric immunobead assay for the detection of BCR–ABL fusion proteins in leukemia patients. Leukemia (2009) 23, 1106–1117.
2. Van Dongen JJM, Van der Velden VHJ, Berendes PB, Van Denderen AC. Recognition of tumour-specific gene products in cancer (Fusion protein bead patent). US6.686.165B2.

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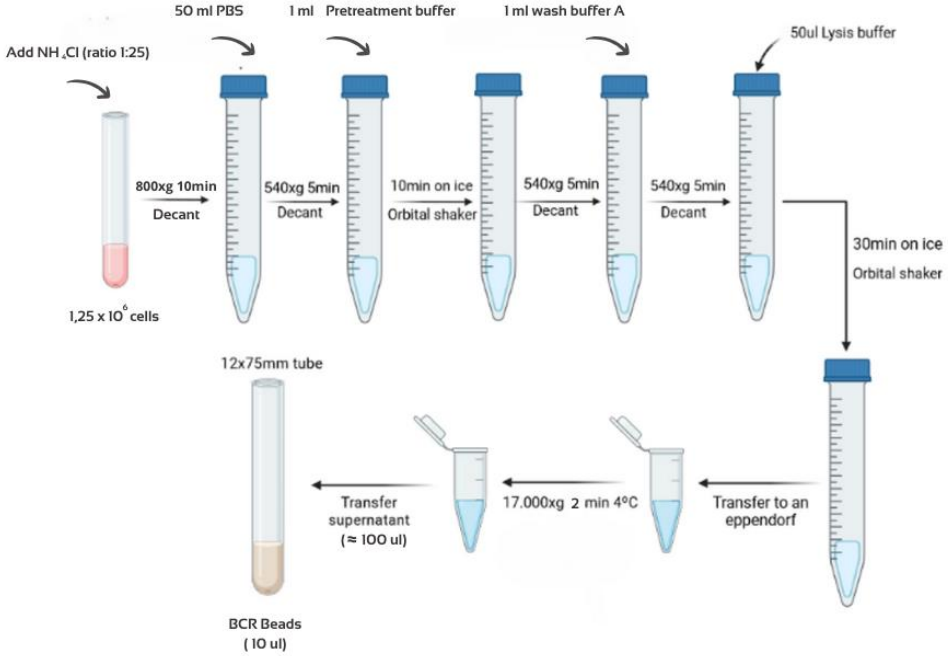
Annex 1: Sample preparation workflow for fusion protein detection.



Annex 2. Immunobead assay protocol.