INTRACELL

Fixation and permeabilization kit for Flow Cytometry

REF	Σ	
INTRA-50T-X	50 test	RUO
INTRA-100T-X	100 test	
INTRA-200T-X	200 test	
INTRA-500T-X	500 test	

1. INTRODUCTION

Staining cells for internal antigens techniques have been devised for permeabilizing cells so that specific antibody can diffuse in and out and access internal antigens. Briefly, cells are fixed with formaldehyde to preserve their morphology, and saponin, a plant glycoside and mild nonionic detergent, is used to generate pores of approximately 8nm in the membrane which allow molecules of up to 200 kDa to pass through. Because of slow diffusion in and out of the cell, longer staining and washing times are required, cell and antibody preparations must be of high quality, and antibody
must be at optimal titre to reduce nonspecific background.

Other treatments can be used. For staining nucleic acid, fixation and permeabilization with alcohol and acetic acid reduces degradation of RNA and DNA. Cells may also be stained with antibody to a cell surface antigen for multicolour analysis.

IntraCell is intended for fixation and permeabilization of cells in suspension for flow cytometry analysis.Immunological detection studies of intracellular antigens on structures such as cytoplasmic and/or nuclear enzymes, requires the permeabilization of the cell membrane in order to allow interaction of the antibody with its intended target. In order to allow recognizable cellular integrity after permeabilization a fixation step involving cross-linking or denaturation is required. This is usually accomplished by aldehyde or alcohol fixation followed by detergent permeabilization of the cell membrane. Labeling of cell surface antigens with antibodies prior to the fixation step is possible thus allowing simultaneous surface antigens phenotyping with internal antigen expression in multiparameter fluorescent cytometric analysis.

IntraCell is developed for use with all flow cytometers and reagents remains intact the cell surface marker expression and the cell properties of FSC and SSC.

2. MATERIALS PROVIDED

Reagent A: Fixative solution

Reagent B: Permeabilization solution

3. MATERIALS DON`T PROVIDED

PBS Working Solution. IX Phosphate- buffered saline solution (PBS) + 0,1 NaN3 + 4% BSA.

4. APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2°C-8°C. Do not use after the dateindicated.

Once the vial is open, the product is stable for 90 days.

5. 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

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

6. WARRANTY

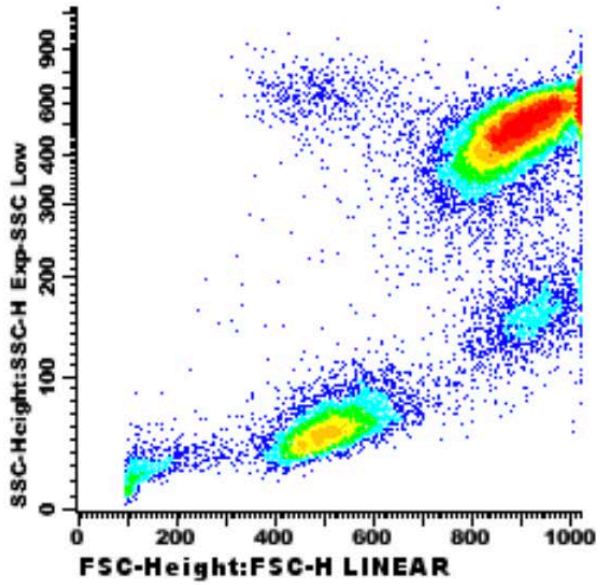
Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price

7. RECOMMENDATIONS AND WARNINGS

a) The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded.These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR IN VITRO DIAGNOSTIC USE.
j) For professional use only.
k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

8. PROTOCOL FOR SAMPLE PREPARATION AND INTRACELLULAR STAINING

1. Pipette 50 µl of cell suspension to be analysed (up to 106 cells) into each tube.
2. For each sample, add an appropriate volume of conjugated antibody directed to the cell surface antigen of interest and the appropriate isotype control. Incubate for 15 minutes in the dark at room temperature. (This step is only necessary is you want to perform a direct immunofluorescence staining for a cell surface antigen)
3. Add 100 µl of IntraCell Reagent A, (Fixative), to each tube. Mix gently.
4. Incubate for 15 minutes at room temperature.
5. Wash once in 2 ml PBS Working Solution IX.
6. Centrifuge for 5 minutes at 300 xg, then aspirate supernatant, leaving approximately 50 µl of fluid and vortex to ensure that the cell pellet are in suspension.
7. Add 100 µl of IntraCell Reagent B (Permeabilization), to each tube. Add the appropriate volume of conjugated intracellular antibody specific for the intracellular antigen and the appropriate isotype control.
8. Incubate for 15 minutes in the dark at room temperature.
9. Wash once in 2 ml PBS Working Solution IX.
10. Centrifuge for 5 minutes at 300 xg, then aspirate supernatant, leaving approximately 50µl of fluid and vortex to ensure that the cell pellet are in suspension.
11. Resuspend the cell pellet in 0,5 ml of 1% paraformaldehyde solution or an appropriate fluid for flow cytometric use, and store in the dark at 2-8 °C. Fixed cells should be analyzed within 24hours.



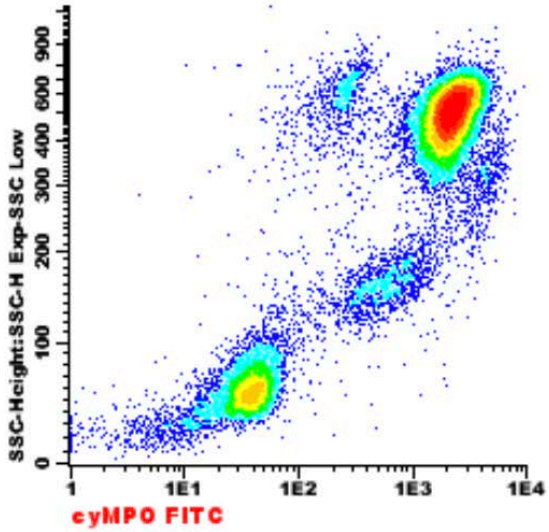


Figure 1. Intracellular FITC mouse anti-human myeloperoxidase (MPO) vs side (SSC) scatter of a normal blood sample.

9 INTRACELLULAR PROTEINS EVALUATED

Enzymes	Myeloperoxidase, Carboxipeptidase, Granzyme B[3], Perforine[3]
Cytoplasmatic CD molecules	CD3, CD13, CD22, CD62P, CD63, CD68, CD79a
Nuclear proliferation markers	BrdU, Ki-67[7], PCNA, TdT
Oncoproteins	Bcl-2, c-Myc, p53[9]
Cytokeratines	CK19[6]
Caspases	CASP3[8]
Cytokines and cheokines	IFN-γ, TNF-α, IL1-β, IL-2, IL-4, IL-10, IL-12, IL-13, IL-16, RANTES
Immunoglobulins	IgA, IgG, IgD, IgM, kappa, lambda
Other molecules	ZAP-70, cyclins, transfected cells, intracellular parasites [4,5,7], lamin A / C7, MDR (multidrug resistance)[1]

10. SENSIBILITY

The quality of each batch of intracell is determined by fixation and permeabilization of several samples of peripheral blood from healthy donors with some of the markers mentioned above and the corresponding comparison of the characteristics of size and complexity of the preparation of leukocyte processed.

11. TROUBLESHOOTING

a) **Most conjugated antibodies are suitable for use together** with IntraCell. However, there are some antigen determinants that are sensitive to the fixation with formaldehyde. And optimal fixation time or formaldehyde concentration, should be determinate for each antibody conjugated.

b) The fixation/permeabilization reagent (INTRACELL) contained paraformaldehide and their cross-linking propriety didn't allow RNA extraction.

c) In the staining procedure, particular attention should be paid to the incubation times.

12. REFERENCES

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13. SYMBOLS

	Fluorochrome
REF	Product reference
Σ	Content for <n> analysis
	Regulatory Status
	Description
RUO	Research Use Only
	Manufacturer

14. MANUFACTURED BY

IMMUNOSTEP S.L.
Address: Avda. Universidad de Coimbra, s/n
Cancer Research Center (C.I.C)
Campus de Unamuno
37007 Salamanca (Spain)
Telf./fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com