

IntraPrep™

REF A07803
150 tests ; 6 x 5 mL
2 x 0.1 mL / test



Leucocytic
Permeabilization Reagent



ENGLISH	Reagent 1	Reagent 2
	Fixation agent	Permeability agent
Formulation	Liquid	Liquid
Active substance	Formaldehyde	Saponine
Volume	5 mL	5 mL
Number of vials	3 vials	3 vials
Volume per test	100 µL	100 µL

USE

IntraPrep consists of two ready-to-use reagents, which induce permeability in the cytoplasmic membrane of leucocytes for the demonstration of intracellular antigenic determinants by means of monoclonal fluorescent antibodies. IntraPrep is used to prepare biological samples for analysis by flow cytometry. It has been optimized in order to minimize the non-specific staining in this type of analysis (1 – 4).

PRINCIPLE

As a first step, cells are fixed with reagent 1. After washing, permeability is induced with reagent 2 and remaining erythrocytes are lysed. During this stage, the cells are brought into contact with conjugated monoclonal antibodies specific for intracellular antigenic determinants. The leucocytes are then analyzed by flow cytometry.

The demonstration of surface antigenic determinants nevertheless remains possible. In this case, the specific conjugated monoclonal antibodies are incubated before fixation.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the localization of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow-angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

STORAGE AND STABILITY

IntraPrep is stored at 18 – 25°C.

Stability of closed vial: see expiry date on vial.

Stability of opened vial: the reagent is stable for 90 days.

PRECAUTIONS

- Do not use the reagent beyond the expiry date.
- Do not freeze.
- Minimize exposure to light.
- Avoid microbial contamination of the reagents, or false results may occur.
- Reagent 1 contains formaldehyde. Formaldehyde is toxic and allergenic. It is thought to be a carcinogenic agent. Never pipette by mouth and avoid all contact with the skin, mucosae, eyes and clothing.
- Reagent 2 contains sodium nitride (NaN₃). It must be handled with care. Do not take

internally and avoid all contact with the skin, mucosa and eyes. Moreover, in an acid medium, sodium azide can form the potentially dangerous hydrazoic acid. If it needs to be disposed of, it is recommended that the reagent be diluted in a large volume of water before pouring it into the drainage system so as to avoid the accumulation of sodium azide in metal pipes and to prevent the risk of explosion.

- All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
- Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

SAMPLES

Venous blood or bone marrow samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant or ACD or heparin.

The samples should be kept at room temperature (18 – 25°C) and not shaken. The sample 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 disposable tips for 10, 20, 50, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- Specific monoclonal antibodies (mAb).
- Isotypic controls.
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PROCEDURES

A - Intracytoplasmic staining

The number of red blood cells present in the sample should be less than 6 x 10⁶ per µL (6 x 10¹²/L). Dilute if necessary in PBS.

The number of leucocytes present in the sample should be less than 5 x 10³/µL (5 x 10⁹/L). Dilute if necessary in PBS.

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed with the negative control corresponding to the specific stain selected.

- Add 50 µL of the test sample into the test tube and into the control tube.

- Add 100 µL of reagent 1 to the two tubes. Vortex vigorously immediately after each addition.
- Incubate for 15 minutes at room temperature (18 – 25°C).
- Add 4 mL of PBS to each tube.
- Centrifuge for 5 minutes at 300 x g at room temperature.
- Remove the supernatant by aspiration.
- Add 100 µL of the reagent 2 to both tubes. DO NOT VORTEX, leave reagent 2 to diffuse naturally into the cell pellet.
- Incubate for 5 minutes at room temperature (18 – 25°C) WITHOUT SHAKING.
- Shake slowly by hand for 2 to 3 seconds.
- Add 20 µL of mAb (specific for an intracytoplasmic antigenic determinant) to the test tube.
- Into each control tube, add 20 µL of isotypic control.
- Gently vortex tube by tube.
- Incubate for 15 minutes at room temperature (18 – 25°C) protected from light.
- Add 4 mL of PBS to each tube.
- Centrifuge for 5 minutes at 300 x g at room temperature.
- Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 or 1 mL of IOTest 3 Fixative Solution (Ref. A07800) at its working concentration (1X). Thus fixed, the preparations can be stored between 2 and 8°C away from light for 24 hours.

B - Intracytoplasmic and membranous staining

The number of red blood cells present in the sample should be less than 6 x 10⁶ per µL (6 x 10¹²/L). Dilute if necessary with PBS.

The number of leucocytes in the sample should be less than 5 x 10³/µL (5 x 10⁹/L). Dilute if necessary with PBS.

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 negative control corresponding to the specific stain selected.

- Add 50 µL of the test sample to the test tube and to the control tube.
- Add to the test tube 20 µL of mAb (specific for a membranous antigenic determinant)
- To each control tube, add 20 µL of isotypic control.
- Gently vortex tube by tube
- Incubate for 15 minutes at room temperature (18 – 25°C) protected from light.
- Add 100 µL of reagent 1 to the two tubes. Vortex vigorously, immediately after each addition.

7. Incubate for 15 minutes at room temperature (18 – 25°C).
8. Add 4 mL of PBS to each tube.
9. Centrifuge for 5 minutes at 300 x g at room temperature.
10. Remove the supernatant by aspiration.
11. Add 100 µL of the reagent 2 to both tubes. DO NOT VORTEX, leave reagent 2 to diffuse naturally into the cell pellet.
12. Incubate for 5 minutes at room temperature (18 – 25°C) WITHOUT SHAKING.
13. Shake slowly by hand for 2 to 3 seconds.
14. Add to the test tube 20 µL of mAb (specific for an intracytoplasmic antigenic determinant).
15. To each control tube, add 20 µL of isotypic control.
16. Gently vortex tube by tube.
17. Incubate for 15 minutes at room temperature (18 – 25°C) protected from light.
18. Add 4 mL of PBS to each tube.
19. Centrifuge for 5 minutes at 300 x g at room temperature.
20. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 or 1 mL of IOTest 3 Fixative Solution (Ref. A07800) at its working concentration (1X). Thus fixed, the preparations can be stored at between 2 and 8°C and away from light for 24 hours.

PERFORMANCE

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the percentage of granulocytes in relation to all of the leucocytes were carried out on blood taken from the same donor. The granulocytes are thus identified, on the one hand, by light diffraction criteria (FS *versus* SS), and on the other hand, by myeloperoxidase expression criteria (MPO). The results obtained are summarized in the following table:

Target	Number	Mean (%)	SD	CV (%)
Granulocytes (FS vs SS)	12	52.4	1.38	2.6
Granulocytes (MPO)	12	55.6	1.6	2.9

INTER-LABORATORY REPRODUCIBILITY

On the same day and by two technicians, 12 measurements of the percentage of granulocytes in relation to all of the leucocytes were carried out on blood from the same donor. The granulocytes are thus identified, on the one hand, by light diffraction criteria (FS *versus* SS), and on the other hand, by myeloperoxidase expression criteria (MPO). Preparations were analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

Target	Number	Mean (%)	SD	CV (%)
Granulocytes (FS vs SS)	12	44.4	0.72	1.6
Granulocytes (MPO)	12	47.2	0.62	1.3

Cytometer n° 2:

Target	Number	Mean (%)	SD	CV (%)
Granulocytes (FS vs SS)	12	44.5	0.90	2.0
Granulocytes (MPO)	12	46.2	0.76	1.7

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. Certain antigenic determinants can be sensitive to formaldehyde or to saponine. Each laboratory must validate the conditions for use of monoclonal antibodies.
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. This reagent has been optimized so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the ratio of reagent volume/number of leucocytes and erythrocytes in every test.
5. In the case of hypercellularity, dilute the specimen in PBS so as to obtain less than 5×10^9 leucocytes / L and less than 6×10^{12} red blood cells / 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, for example) prior to staining.

MISCELLANEOUS

See the Appendix for Examples and References.

TRADEMARKS

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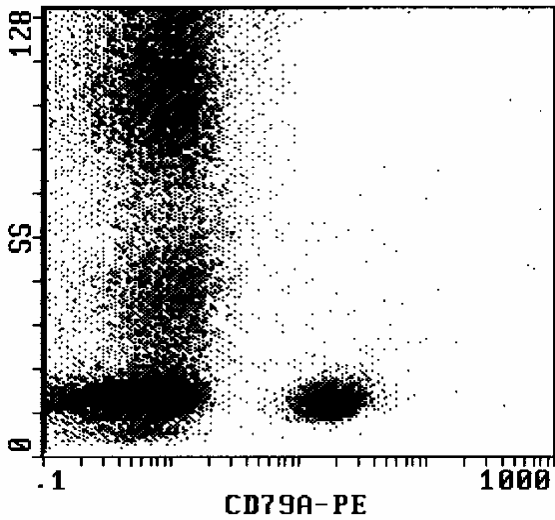
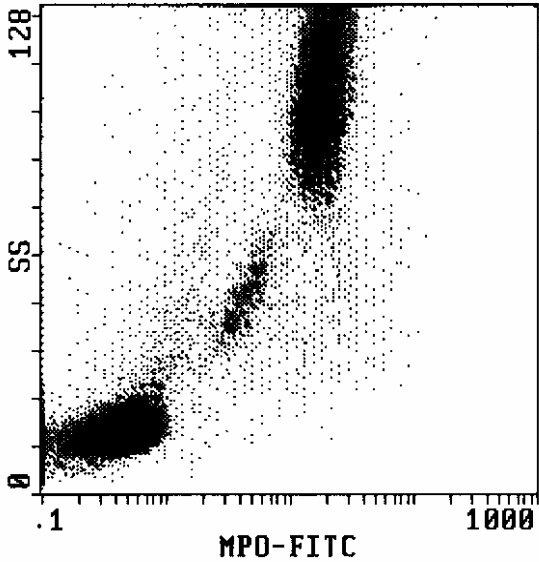


APPENDIX TO REF A07803

EXAMPLES

The graphs below are ungated biparametric representations (Side Scatter vs Fluorescence Intensity) of normal whole blood sample. Intracellular staining is with MPO FITC-conjugated monoclonal antibody and with CD79a PE-conjugated monoclonal antibodies.

Acquisition and analysis are with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ software.



REFERENCES

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