FaP-KIT 200 Tests

FIXATION & PERMEABILIZATION of cells for intracellular staining

MabTag GmbH

Ref.: FaP-200KIT

1 test = 100µl FIX + 100µl PERM (200 tests = 2x 20ml)

For research only. Not for use in diagnostic or therapeutic procedures.

MabTag's **FaP-Kit** contains appropriate ready-to-use reagents sufficient for RBC-lysis, mild fixation and inducing of permeability in cytoplasmatic membranes of leucocytes and immunologic detection of antigens by means of antibodies and subsequent analysis by flow cytometry or microscopy.

MabTag's FaP-Kit features

- *Fast and efficient – whole procedure can be performed in one hour*
- *Enables simultaneous detection of both intracellular and cell surface markers*
- *Softly fixed cells with conserved flow cytometric scatter properties*
- *Specific formulation for reducing background and simultaneous RBC-lysis*
- *Quality control assures minimization of lot-to-lot variations*

Content	Volume per test	Active compound	Storage	Stability
20 ml FIX	100 µl	Formaldehyde	10 – 25°C	Closed vials: 3 years after delivery
20 ml PERM	100 µl	Detergent	10 – 25°C	Opened vials: 6 months

Principle

In a first step the cells are fixed by using **FIX** reagent. Subsequently the cells are permeabilized and erythrocytes are lysed by using PERM reagent. Intracellular antigen detection goes on after permeabilization step. Surface marker detection goes on prior to the fixation step or parallel to the intracellular detection step provided that antibodies against surface markers are able to detect fixed surface markers as well.

Procedure

Step	Incubation	Procedure
Sample		Add 100 µl of the sample (blood, bone marrow sample or other cell suspension) into your test tube (≥ 5ml). The sample must be completely on the bottom of the tube.
Detection of surface markers	20 min / RT	For immuno-staining add a sufficient volume (usually 1-20 µl depending on the manufacturer) of antibody solution into the sample, vortex and incubate.
Fixation	15 min / RT	Add 100 µl of **FIX** reagent to the test tube, vortex and incubate. Several short vortex cycles within the incubation may optimize the results.
	10 min / RT	Add 2.5 ml 1xPBS to the test tube and incubate without any movement. The incubation time of 10 min is optional – each customer should experimentelly check, wether it is advantageous for detection of the considered intracellular antigen
Washing		Centrifuge for 5 min at 400g → remove **completely** the supernatant
RBC-Lysis + Permeabilization + Detection of intracellular antigens	25 min / RT	For intracellular immuno-staining add a sufficient volume (usually 1-20 µl depending on the manufacturer) of antibody solution **into** the sample / pellet.
		Add 100 µl of **PERM** reagent to the test tube, shake gently approx. 5 sec and incubate. Several short vortex cycles within the incubation may optimize the results. Note: This kit is optimized for a sample of 100µl blood. It can be necessary to adjust the volume of **PERM** reagent if you handle other samples. For example: a mixture of blood and culture medium at a ratio 1:1. In such a case there are only 50µl blood in 100 µl sample. Sometimes you obtain more reliable results by addition of 50µl of **PERM** reagent to the pellet instead of 100 µl.
Washing		Add 2.5 ml PBS and centrifuge for 5 min at 400g → remove **completely** the supernatant
		Resuspend the pellet in approx. 0.5 - 1ml 1xPBS for analysis

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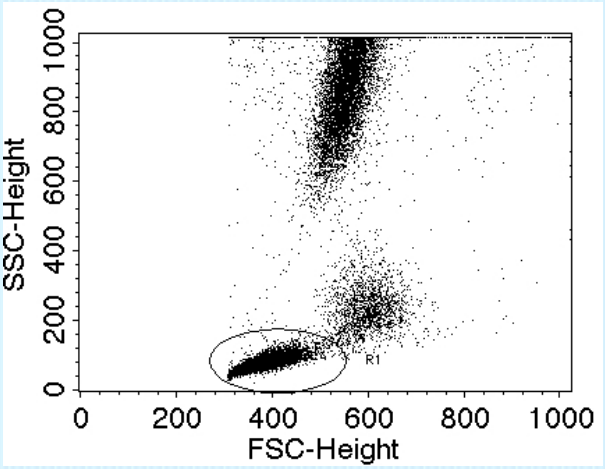
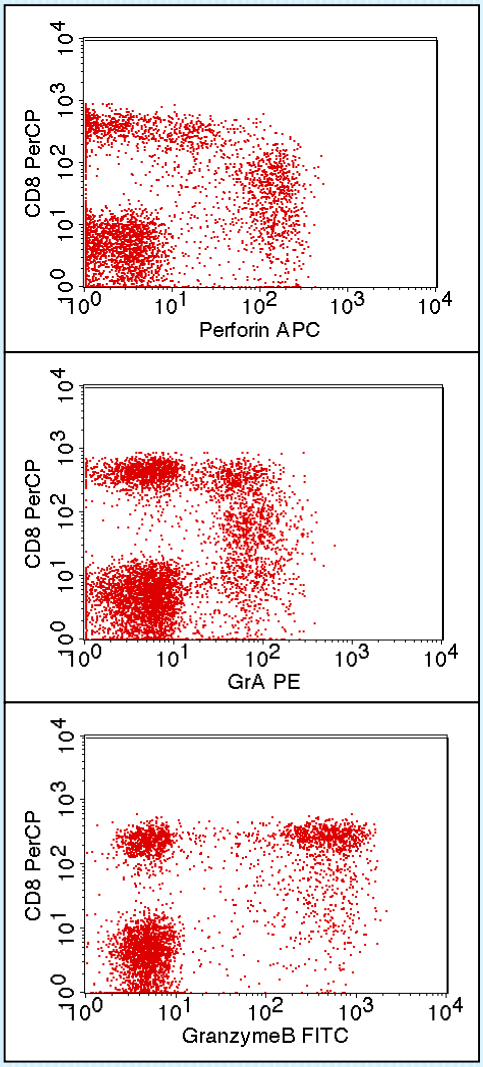
Hints & Limitations

- This kit is designed only for professional and experienced laboratory personnel which is well-versed in good laboratory practices.
- For correct cytometric analysis the flow cytometer must be perfectly aligned and compensated.
- Some antigens can be too sensitive to aldehyds or detergents.
- Some detection antibodies are not sufficient for fixed antigens.
- The technical insert has to be read completely before running the procedure.
- Lysis of erythrocytes of patients suffering from certain diseases may be incomplete or impossible
- For certain samples it may be necessary to isolate mononuclear cells (e.g. by means of Ficoll gradient centrifugation) prior to the fixation/permeabilization procedure
- Antibody staining at higher temperatures and longer incubations times can lead to higher non-specific background signal

Caution

For professional users only. MabTag's FaP-Kit solutions contain fomaldehyde and sodium azide which are HARMFUL. Formaldehyde and sodium azide are toxic, allergenic and suspected carcinogens. Never pipette by mouth and avoid contact with eyes, skin and clothing. Users must be well instructed in the accurate working procedure, the dangerous properties of the product and the necessary safety instructions. Please refer to the Material Safety Data Sheet (MSDS) for additional information. Dispose product remainders according to local regulations.





Example:

Intrazellular detection of ***granzyme A, granzyme B*** and ***perforin*** in whole blood lymphocytes.

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