## KIT COMPOSITION

### PerFix-nc Kit - PN B10825 - 75 tests

Storage at room temperature (18-25°C)
12-month shelf life at batch manufacturing

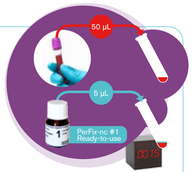
**Components:**

1. **PerFix-nc Buffer 1** Fixative Reagent
2. **PerFix-nc Buffer 2** Permeabilizing Reagent
3. **PerFix-nc Buffer 3** Final 10X Solution

## PerFix-nc PROCEDURE

PerFix-nc *STRAIGHT*
Permeabilization and Staining Procedure

### 1




a. Pipet 50 µL of blood sample into the bottom of each relevantly labeled tube.

b. Pipet 5 µL of Buffer 1 (Fixative Reagent) to each tube.
*Alternatively use 25 µL if better for the intracellular antigen(s).*

c. Vortex immediately (instantly after Buffer 1 addition) and incubate for 15 min at room temperature (18-25°C).

### 2


d. Vortex again the fixed blood and add 300 µL of Buffer 2 (Permeabilizing Reagent) to each tube.
Vortex.

e. Add immediately to each tube the relevant volume (e.g. 20 µL) of fluorochrome-conjugated antibodies against intracellular epitopes and surface molecules.

f. Vortex and incubate in the dark for 15-30 min at room temperature.

Note regarding steps d and e:
Alternatively, the antibodies can be pre-mixed into Buffer 2 (Permeabilizing Reagent) and added altogether at the end of the fixation incubation.

### 3

g. Add 3 mL of the Final 1X Reagent (prepared from the 10X concentrated Final Solution) to each tube.
Vortex immediately.

h. The sample is now ready for analysis on a flow cytometer.


**PerFix-nc has been developed to:**

- Simplify the workload necessary for the sample preparation
- Enhance the signal-to-noise ratio of intracellular staining

**Accurate detection of both intracellular and extracellular epitopes are obtained, while:**

- There are no washing steps through the whole STRAIGHT procedure! (only a final wash step is described as optional)
- Several surface markers can be added together with the intracellular markers and incubated simultaneously during the permeabilization step (Buffer 2 incubation)
- Total duration of the procedure and total workload are similar to current procedures for surface staining (e.g. VersaLyse + Fixative No Wash), and much shorter than competitive permeabilization procedures
- Automation of the PerFix-nc STRAIGHT procedure is rendered possible thanks to the removal of the washing steps

- Less than 15 minutes actual hands-on time
- No centrifuge, No wash straight procedure
- Still versatile, with optional wash
- Adaptable to specific antigen detection requirements
- Incubate surface and intra markers simultaneously
- Room temperature (no water bath or ice required)
- Remarkable cell recovery and gate purity
- Best-in-class for RMFI

Ordering Information:

PerFix-nc 75 tests Part Number B10825

RUO: For Research Use Only. Not for use in diagnostic procedures.


BECKMAN COULTER



For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at www.beckmancoulter.com

B2012-12987



BR-16586A - Outside US Version

Surface & intracellular staining in a single step


PerFix-nc Kit (no centrifuge assay kit)

SPEED
OPTIMAL QUALITY
NO CENTRIFUGE

*For more information go to www.beckmancoulter.com*

BECKMAN COULTER

# PerFix-nc

**An innovative tool** for flow cytometric analysis of multiple intracellular epitopes, or both multiple intracellular and cell surface markers, by fixation, permeabilization and staining of human leucocytes from peripheral whole blood, together with erythrocyte lysis, all without centrifugation!

## WORKFLOW AND WORKLOAD TIMING COMPARISON




**Less Than 15 Minutes Work**

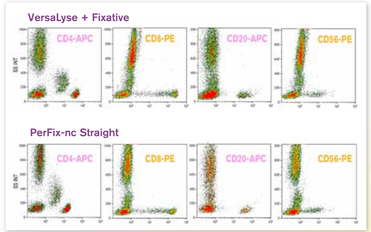
*Take the Right Train!*

## PerFix-nc PROMISES


**Get in touch with your inner cell!**

# PerFix-nc

## PerFix-nc typically does not affect staining patterns or scatters




Comparison of the staining obtained with the VersaLyse + Fixative procedure (reference method for red blood cell (RBC) lysis and white blood cell (WBC) staining) with the PerFix-nc procedure.
Both are used in a no-wash condition, on whole blood from the same donor, with optimized doses of conjugates.

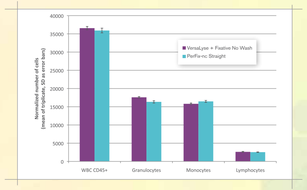
Shown here are CD4, CD8, CD20 and CD56. CD3, CD11b, CD14, CD15, CD16, CD19, CD22, CD25 and CD33 have been tested in the same conditions and show similar results.

***Most surface gating markers can be used in this procedure, together with the intracellular markers, during the permeabilization step.***

## No cell loss with PerFix-nc procedure

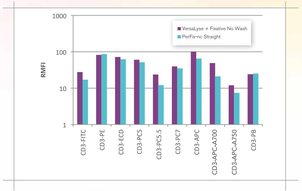
Comparison of the white blood cell (WBC) recovery obtained with the VersaLyse + Fixative procedure (reference method for red blood cell (RBC) lysis and white blood cell (WBC) staining) with the PerFix-nc procedure.

Both are used under no-wash conditions, on whole blood from the same donor (triplicate), with CD45 and CD14 as gating tools.




***All three main leucocyte populations are fully recovered with the PerFix-nc Straight procedure.***

## Compatible with all the dyes from Beckman Coulter portfolio




RMFI as a function of the lysis system
(Recommended dose of mAb: 10 or 20 µL)

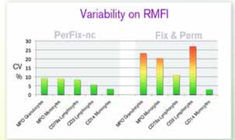
Comparison of the staining obtained with the VersaLyse + Fixative procedure (reference method for red blood cell (RBC) lysis and white blood cell (WBC) staining) with the PerFix-nc procedure.

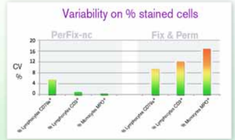
Both are used under no-wash condition, on whole blood from the same donor, with all the available conjugates of the CD3 monoclonal antibody, including Krome Orange (not shown in this graph).

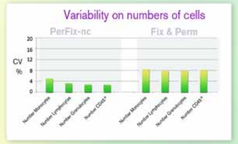
***For all conjugates, there are no significant differences between the two procedures.***
***All Beckman Coulter fluorochromes are compatible with the PerFix-nc.***

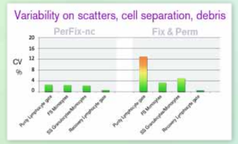
RMFI = relative mean fluorescence intensity.
APC-A700 = APC-Alexa Fluor* 700 ; APC-A750 = APC-Alexa Fluor* 750

## The lowest variability on all parameters



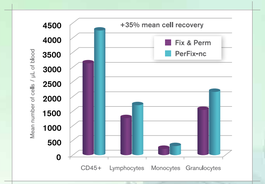

Normal whole blood sample processed 10 times with both methods in a reference laboratory §.
Staining with CD45, CD14, Anti-MPO, CD79a and CD3.

Comparison of the results obtained with the Fix & Perm procedure (Life Technologies) *vs.* the PerFix-nc Straight procedure. Both techniques are used according to the supplier's instructions.

***All the common cytometric quality parameters (RMFI, % of cells, number of cells and scatter patterns) show unprecedented CV values with PerFix-nc!***

***Furthermore, the Straight, no-wash procedure allows a total recovery of cell populations.***

***PerFix-nc is the only permeabilizing product that allows absolute counting of cells!***

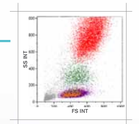


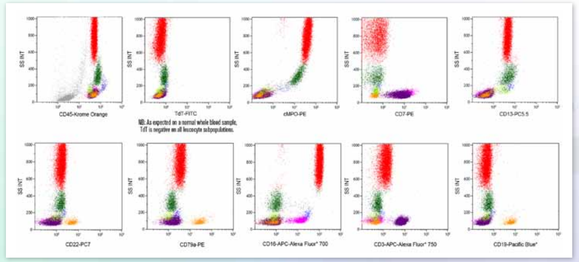

§ Dr Bernard Drénou, Dr Agathe Debliquis, Hematology Laboratory, Emile Muller Hospital, Mulhouse, France.

## Examples of surface and intracellular staining

A representative serie of staining of normal whole blood sample.
All Beckman Coulter-offered fluorochromes are depicted conjugated to various antibodies.

***Top right histogram shows FS/SS pattern emphasizing the state-of-the-art discriminative power between lymphocytes and debris, and between lymphocytes, monocytes, and neutrophils.*** ▶

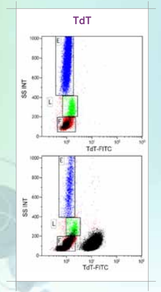


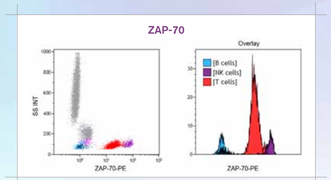

NB. As expected on a normal whole blood sample, TdT is negative on all leucocyte subpopulations.

▲ ***A set of ten SS vs. Fluorescence histograms demonstrating the exceptional quality of the signal obtained whatever the analyzed markers (surface or intracellular) as well as a very low background.***

## Examples of challenging intracellular stainings

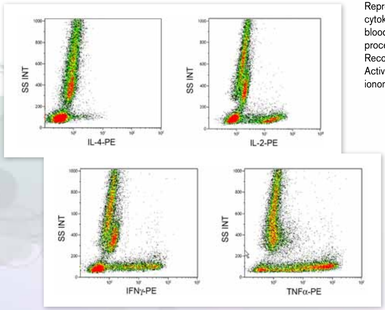



◀ As a nuclear enzyme, TdT can be perceived as a difficult target to reach.
Not with the PerFix-nc Straight procedure as depicted by this SS *vs.* FITC histogram of blood specimens without (top) or with (bottom) TdT expressing lymphocyte subpopulations.




▲ Unprecedented resolution between B, T and NK cells expressing no, medium and high levels of ZAP-70, respectively.
Here the optional final centrifuge step is applied for optimal signal-to-noise ratio.

## Cytokines staining on whole blood

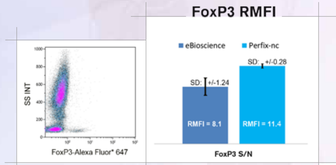



Representative histograms of intracytoplasmatic cytokine stainings on activated normal whole blood cells, using a modified PerFix-nc procedure (high fixative and final centrifugation). Recommended blood anticoagulant is Heparin. Activation of cells is done with PMA + ionomycin + BrefA.

PMA = Phorbol 12-myristate 13-acetate
BrefA = Brefeldin A

***Tremendous dynamic range of stainings on whole blood samples without the need of the tedious PBMC preparation.***

## FoxP3 staining: Easy and robust PerFix-nc procedure







Normal whole blood stained with Alexa Fluor* 647 conjugated Anti-FoxP3 (clone 259D), using a modified PerFix-nc procedure (60 minutes staining followed by an extra PBS wash) as compared to the standard eBioscience procedure.