iba Solutions For Life Sciences

Cell Selection & Expansion

Application Note

FABIAN® - TRACELESS POSITIVE LYMPHOCYTE ISOLATION

Fully reversible, non-magnetic cell selection directly from whole blood

A standard problem in cell preparations is limited purity and recovery, which causes variability in the quality and potency of cell products. Highly pure populations can be obtained best using positive selection techniques. However, the latter are often based on high affinity antibodies whose usage causes unfavorable effects like strong and almost irreversible binding to cells, cell stimulation as well as receptor blockade.

IBA's newly developed FABian® cell selection device is a fully automatic bench top instrument for quantitatively selecting cells of interest in high yields and purity from whole blood, buffy coat or other blood preparations. FABian® employs IBA's unique Strep-tag®/Strep-Tactin® platform for traceless affinity cell selection (Fab-TACS®) of cells. Fab-TACS uses immune affinity chromatography based on CD-specific Fab-fragments for reversible capture and release of target cells (**Fig. 1**). The innovative Fab-TACS procedure[1] delivers label-free, non-activated target cells in a standardized manner of highly reproducible quality without laborious density gradient centrifugation.

This application note compares composition and quality of PBMC fractions either isolated with conventional density gradient centrifugation or with FABian® CD81⁺ Fab-TACS®. Therefore, we determined the amounts of all cell subpopulations as well as their viability. In addition, we tested for cell activation potentially caused by the selection process.

The relative amount of leukocytes isolated from 9 ml peripheral blood using IBA's CD81⁺ Fab-TACS® procedure is on average 1.7-fold higher than with conventional density centrifugation (**Fig. 2A**).

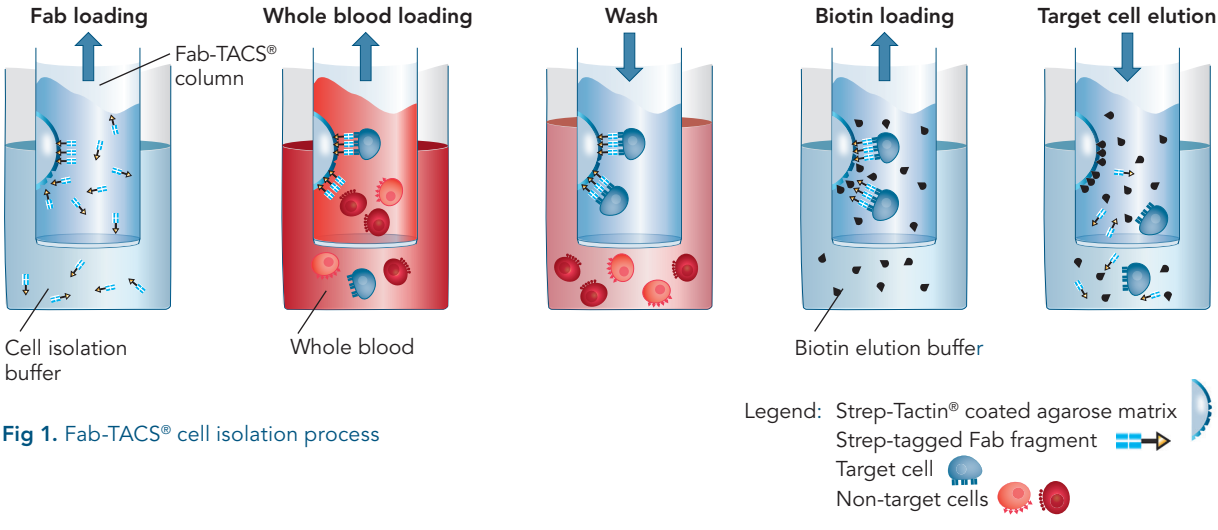
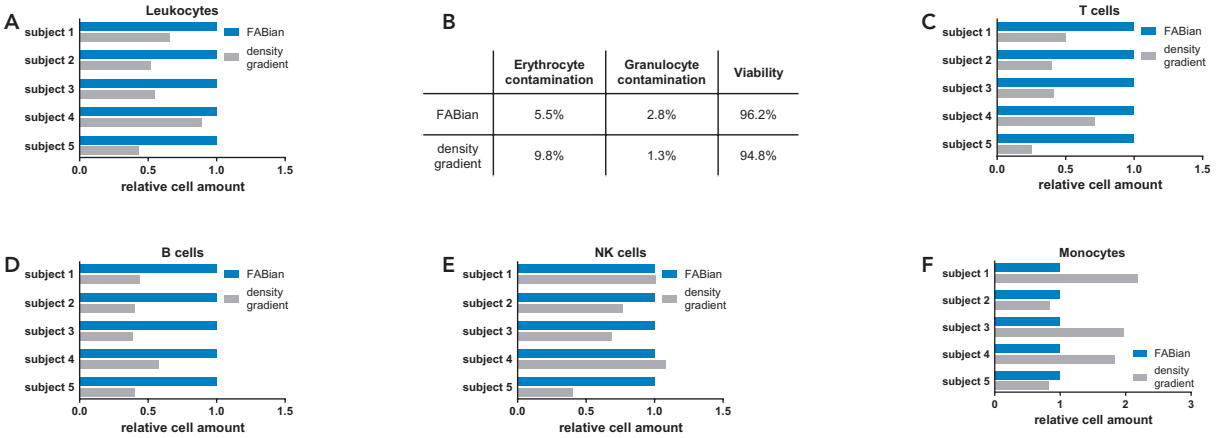


Fig 1. Fab-TACS® cell isolation process



	Erythrocyte contamination	Granulocyte contamination	Viability
FABian	5.5%	2.8%	96.2%
density gradient	9.8%	1.3%	94.8%

Figure 2. Comparison of standard density gradient centrifugation with FABian® CD81 Fab-TACS®
Isolated cells from five individuals were counted on a CytoFlex flow cytometer. Dead cells (DAPI+) were excluded from analysis.

The effective washing steps during the Fab-TACS® positive cell selection process significantly reduces erythrocytes and eliminates thrombocytes while maintaining an excellent viability (**Fig. 2B**). Granulocytes appear due to co-isolation of CD81⁺ eosinophils[2]; however, these usually unwanted cells are as little present as compared to the density gradient isolation.

Lymphocyte yields are significantly higher in the isolated CD81⁺ PBMC-like fraction including T cells, B cells or NK cells (**Fig. 2C-E**). The monocyte fraction is underrepresented in the Fab-TACS® procedure (**Fig. 2F**) due to a lower expression of the CD81 surface marker on this subpopulation[3].

To monitor a putative cell activation, we focused on the most prominent indicators for activation in T cell and B cell populations (IL-2, TNF and IFN-γ) and measured their secretion after 24 h of cell cultivation (**Fig. 3**). Despite positive selection, CD81⁺ fractions isolated with FABian® do not show any secretion of the selected cytokines. Moreover, a subsequent stimulation of the isolated cells, resulted in stronger IL-2, TNF and IFN-γ secretion in FABian®-isolated cells, indicative of a high immunocompetence.

In summary, IBA's novel automated cell selection technology provides an effective and reproducible method for the isolation of cells in high yields, purity and viability. For more details see the recent paper by Pelàk et al.[1]

Methods

Materials and methods are available as supplementary materials on www.iba-lifesciences.com/fabian

References

[1]Pelák O, et al. (2016). Cytometry Part A, DOI: 10.1002/cyto.a.22918.
[2]Tedder TF, et al. (1995). Leukocyte Typing V: White Cell Differentiation Antigens. Vol.1. New York, NY: Oxford University Press; 1995:684-688.
[3]Pugholm LH, et al. (2016). J Immunol Res. Volume 2016, Article ID 6391264.

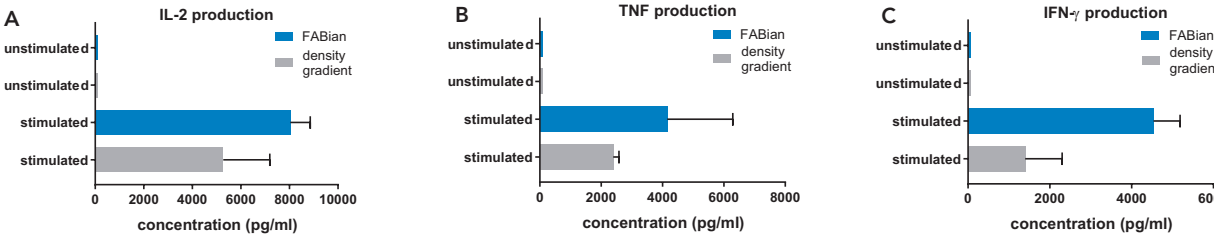


Figure 3. Cytokine production of PBMC-like fractions isolated with FABian® CD81⁺ Fab-TACS or conventional density gradient.
Isolated cells from three individuals were analyzed by flow cytometry and cultured for 24 h under sterile conditions. Cytokine secretion of IL-2 (A), TNF (B) and IFN-γ (C) were measured using CBA and flow cytometry. PMA/ionomycin was added as a positive control for cell stimulation. In all cases, the determined values for the unstimulated cells were below detection level. The bars were only added for graphical reasons.

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