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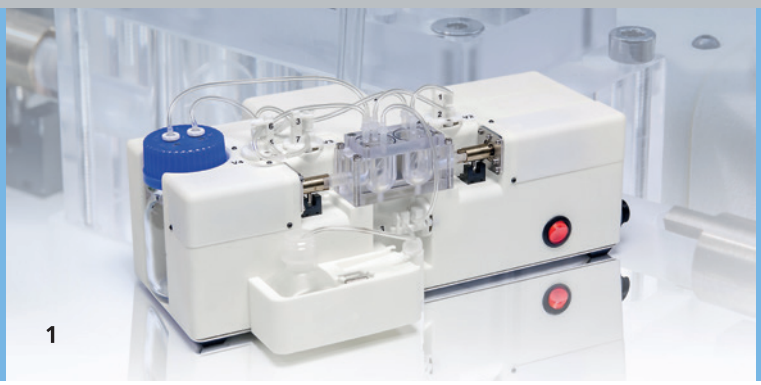

1 *Automated MACS unit for negative-positive selection.*

MAGNETIC CELL SEPARATION UNIT

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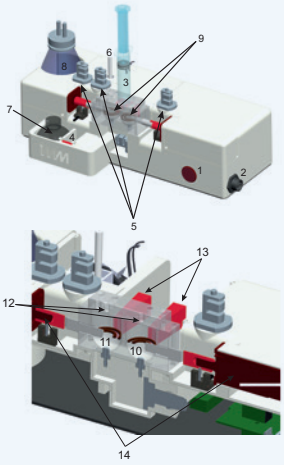
IN COOPERATION WITH

AdnaGen AG

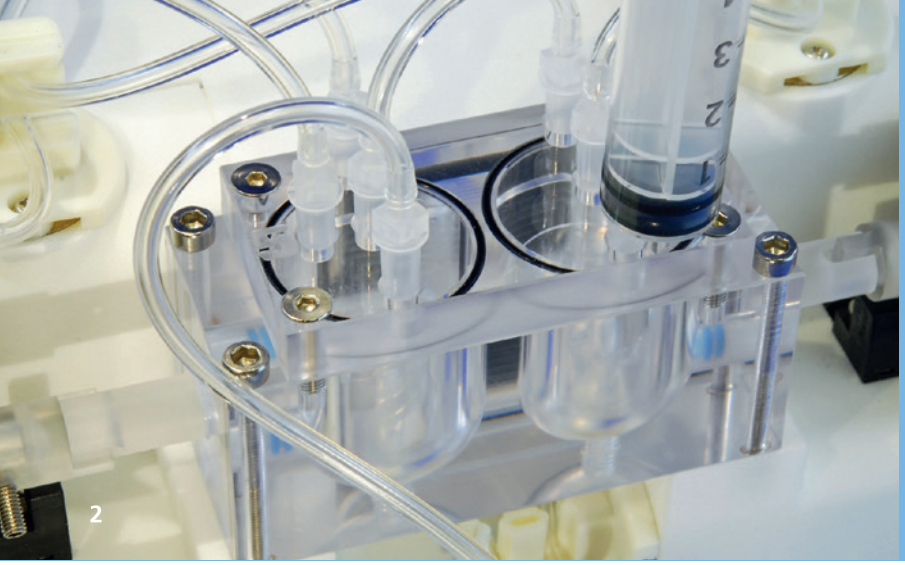
Introduction

The enrichment of targets from biological sample material is a great challenge in biomedical analysis. Magnetic cell separation (MACS) is a widely spread used method to separate target cell types by magnetic beads. This method is especially useful for isolation of rare cells (i.e., circulating tumor cells, stem cells, fetal cells, etc.) from high sample volume with high nontarget cell background. Unspecific adsorbed and, hence, falsely enriched nontarget cells lead to a disturbing background signal. Due to the repetitive selection/separation steps, the complete MACS procedure is simple but expensive and time consuming. The automated MACS process not only minimizes the hands-on time drastically but more importantly lowers the risk of lost target cells. Furthermore, the reproducibility and reliability of the separation method itself is increased since manually processing is the most prominent source of failure and costs.

Schematic overview of the automated stand-alone MACS unit.



1: On/off switch
2: USB port
3: Syringe for sample insertion into the system
4: Magnetic interface
5: Pitch valves
6: Interface to peristaltic pump
7: Waste
8: Buffer reservoir
9: Stirrer (engine driven)
10: Chamber 1
11: Chamber 2
12: Luer adapter
13: Magnets for bead accumulation
14: Motors



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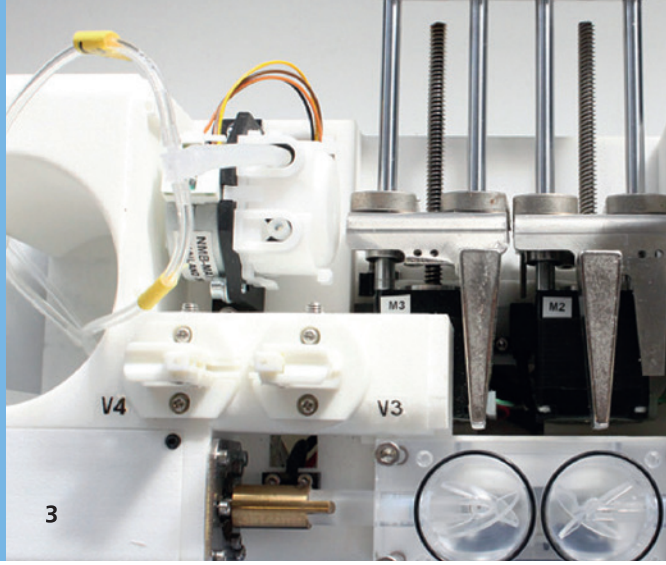

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Instrument Setup

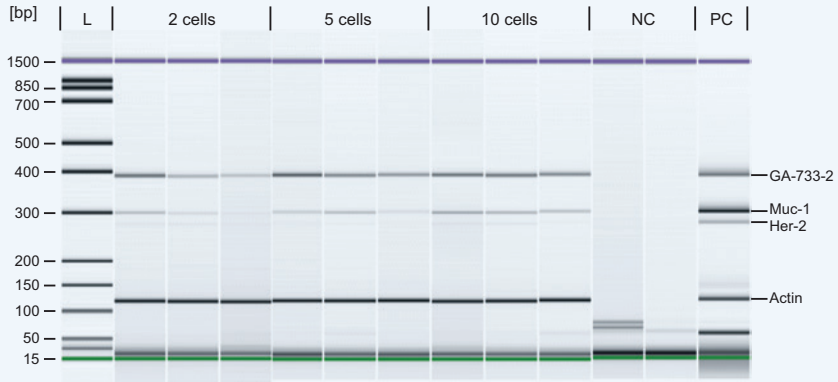
The basic module of the MACS demonstrator consists of an active mixing chamber. Separation and solution exchange are realized fully automated via tube valves. To realize a negative and a positive selection, a two chamber module (MACS unit) has been developed. For separation, several milliliters of whole blood are added into the first chamber, where the beads are pre-stored. The blood and the beads are mixed subsequently to get optimal formation of cell-bead complexes. In the following step, adjustable external magnets attract the magnetic beads. The unbound leftover is transported into the waste. The cell-bead complexes are now repeatedly washed by getting resuspended and reattracted with the magnets. After elution, the solution with the complexes is transported into the second mixing chamber for negative selection. The isolated cells can be sequentially transported into a microfluidic LoC system or to a sample tube, respectively.

Results

For the experimental evaluation of the MACS unit, the double selection and the positive selection alone are performed. In all cases, varying amounts of human breast cancer cells (MCF-7) were spiked into 5 ml of different media (cell culture medium, whole blood, and buffy coat). For the isolation experiments, 4.5µm beads were used. The cell isolates were analyzed by RT-PCR. For the determination of the nontarget background, the isolates were also tested for CD45. The whole procedure takes one hour for positive selection and one and a half hours for combined negative and positive selection.
A detection down to 2 cells within 5 ml whole blood was possible. After the cell isolation procedure in the MACS unit, about 2000 cells were detected as unspecific background.

Summary

A small fully automated MACS demonstration unit has been established and a detection down to 2 cells within 5 ml sample is successfully reproducible. Based on a reusable polymer incubation chamber with integrated mixing paddle in a compact custom made instrument, the unit is a reliable standalone solution for enrichment and isolation of rare cells. The reliability of the demonstrator has been proven either as fully autonomously working MACS unit or as part of a sample-to-answer platform in several R&D projects at Fraunhofer IMM as well as at several other labs.



L: ladder NC: negative control PC: positive control

Fig. 3: Successful positive selection of 2, 5, and 10 target cells from 5 ml whole blood. Analyses of isolated cells by using multiplex PCR (AdnaGen Kit).

2 *Detailed view of the two mixing chambers including the fluidical ports for puffer exchange.*

3 *Magnetic interface in detail.*