Fraunhofer IMM

Fraunhofer Institute for Microengineering and Microsystems IMM

Microfluidic systems

CTCelect: Isolation of single rare cells from human blood

Quick Facts

- fully automated isolation of CTCs from 7.5 ml whole blood sample
- combined two-step process:
  step 1: immunomagnetic enrichment
  step 2: isolation/dispensing
- microfluidic cartridge technology for isolation/dispensing step
- precision cell dispensing directly in wells of a microtiter plate in 1-3 µl droplets
- excellent recovery rate ~90 % for each process step (model system of spiked MCF7 cells)
- high purity of the isolated cells for further analysis (only 1 out of 10 CTCs dispensed with a leukocyte)
- flexible platform technology; assay adaptable to a specific application by the user (choice of reagents, programming of process steps)
- independent use of the two modules possible

Keywords: Cell isolation, single cell analysis, liquid biopsy, CTC, microfluidics

Liquid biopsy for future cancer therapy

Future cancer therapy is expected to rely strongly on "liquid biopsy" which is based on the analysis of liquid samples (e.g. blood) of cancer patients. Tumor cells circulating in the blood of cancer patients (CTCs) shall be used to gain detailed molecular diagnostic information on tumor subtypes to tailor the therapeutic intervention for each individual patient ("personalized medicine"). However, this requires methods in order to reliably detect and isolate the very rare CTCs from billions of blood cells with high sensitivity and specificity.

The CTCelect instrument

CTCelect is a fully automated instrument which directly links clinical blood sampling with state-of-the-art single cell analytics. CTCelect starts with a raw human blood sample in a standard sampling tube, enriches the CTCs, detects them in a continuous flow and dispenses single CTCs selectively into wells of a standard microtiter plate resembling the starting point for single cell analysis. The corresponding assay can be adapted by the user with an intuitive graphical user interface.

The workflow is implemented in two main functional modules of the instrument:

1) Enrichment module
Starting with a blood tube carrying 7.5 ml of blood, a fully automated pipetting station enriches the CTCs using specific immuno-magnetic beads, reducing the sample volume to 0.5 ml. In parallel, the CTCs are labelled specifically with a fluorescent antibody (e.g. PE, FITC, quantum dots). The process is based on a carefully tailored reagent kit. Besides the kit reagents, only standard laboratory consumables are required (disposable pipetting tips and tubes).

Up to 11 tubes can be used for the enrichment and labeling process offering the flexibility to select various reagents, buffers, magnetic beads, antibodies, and fluorophores.

2) Isolation module
The pre-enriched sample is transferred automatically to the microfluidic cartridge. An integrated flow cytometry module detects CTCs and triggers the dispensing unit. A second fluorescence channel is available for parallel detection of a second cell type, such as leukocytes, control particles or a second CTC population, labeled with an appropriate fluorescence marker. CTCs are dispensed individually into wells of a microtiter plate. If required, pooling of the dispensed cells is also possible.

The microfluidic cartridge is disposable and for exclusive use in the CTCelect instrument. All processing steps are carried out automatically inside the device. Handling steps are minimized and limited to (re)placing tubes, microtiter plate, microfluidic cartridge and buffer bottles on the workbench.

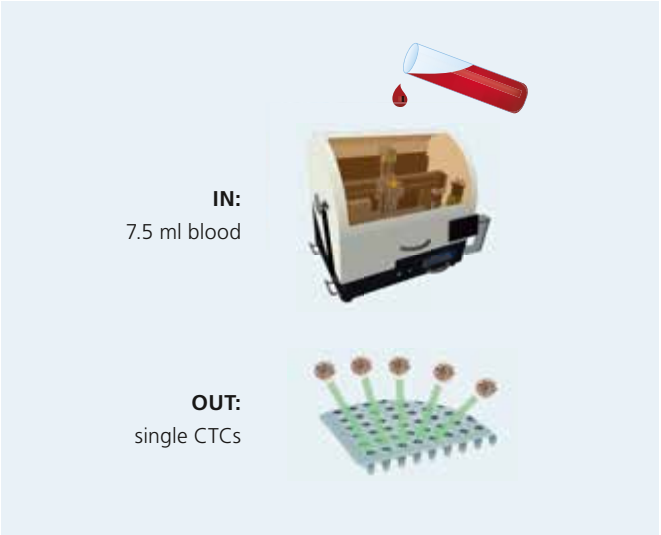
Both modules can either be used in an overall automated process or individually.

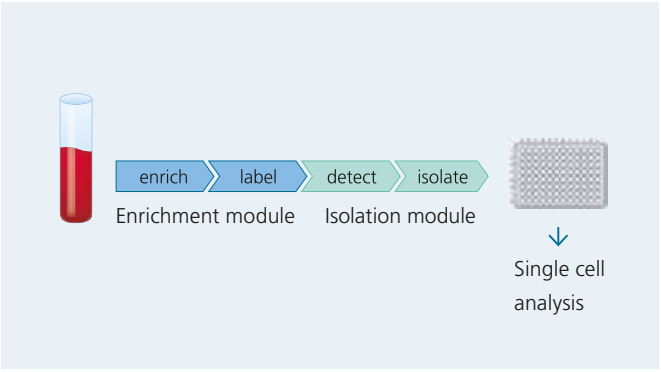
Results on a model system (MCF7 spiked in donor blood)

For each process step (enrichment, detection, dispensing) a recovery rate of ~ 90 % was found, corresponding to a total recovery rate of more than 70 %. The total process run time is about 2.5 h for the enrichment step incl. labeling and about 0.5 h for the isolation step. Downstream single cell PCR has been demonstrated.

Scope of application

- academic and clinical research on tumor cells
- not limited to tumor cells
- handling of EpCAM-negative CTCs using specific antibodies/antibody mix
- isolation of rare cells (fixed or unfixed) from other sample matrices
- enrichment module also applicable for enrichment of exosomes, cfDNA and others from large volumes
- isolation module also applicable for fast single cell dispensing from arbitrary cell populations





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