

Fractionation of Blood Cells

MOTIVATION

A fast, easy, reliable method of isolating certain cells from a blood sample should be based on properties associated with the cells themselves and, ideally, should avoid labelling procedures. Purification of low-abundance species, in particular, benefits from label-free separation methods. Centrifugation and multi-step procedures should be avoided.

SOLUTION

The Cytocon™ 300 instrument can be used for automated separation according to intrinsic properties of the blood cells. In a continuous procedure a "dielectric sieve" distinguishes lymphocytes from red blood cells. Both cell types are collected in two separate fractions. The separation takes place in a closed system and can be performed under physiological conditions.

PRINCIPLE

The separation principle of Cytocon™ is a unique combination of dielectrophoretic force and fluid flow. The nature of the objects contributes to the outcome of the procedure. Differences in type, size or even morphology can be exploited with Cytocon™ separation procedures. Examples are: cells and beads of the same size; cells of different sizes (e. g. mammalian cells and yeast) and nucleated/anucleated cells.

REFERENCE

Müller, T., Schnelle, T., Gradl, G., Shirley, S. G. and Fuhr, G.: Microdevice for cell and particle separation using dielectrophoretic field-flow fractionation; *Journal of Liquid Chromatography & Related Technologies* 2000, 23(1), 47-59.

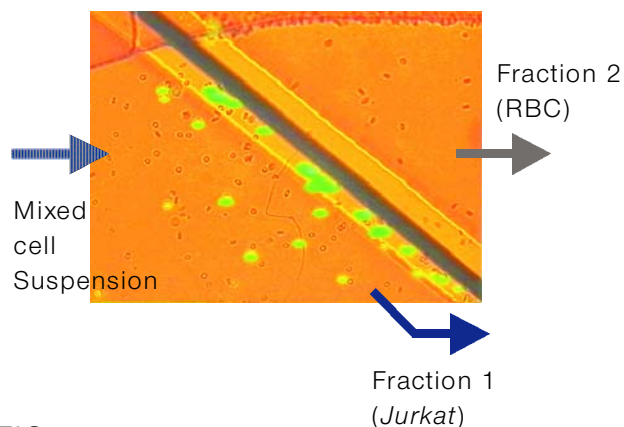


FIG. 1

Separation of calcein-labeled *Jurkat* cells and red blood cells. Only red blood cells can pass the "dielectric sieve" and are collected in fraction 2. The *Jurkat* cells are diverted and are collected in fraction 1.

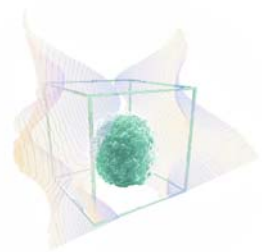
INPUT CELL NUMBER		
total	Jurkat	RBC
6355	161	6194
100%	2.5%	97.5%

FRACTION 1		
total	Jurkat	RBC
345	151	194
100%	43.8%	66.2%

FRACTION 2		
total	Jurkat	RBC
6010	10	6000
100%	0.17%	99.8%

TAB.:

Dielectrophoretic separation of *Jurkat* cells (target) and red blood cells (RBC). The target was diluted in the cell sample to a concentration of 2.5%. The enrichment factor for *Jurkat* cells in fraction 1 was 17.5. The yield of *Jurkat* cells in this fraction was 93 %.



CYTOCON™ 300

APPLICATION
NOTE 4



METHOD

- For a better visualization during the experiment *Jurkat* cells were labelled with 10 μ M Calcein – AM™ (Molecular Probes) and diluted into Cytocon™ Buffer II.
- A blood sample was drawn from a healthy donor by needle puncture and diluted into Cytocon™ Buffer II.
- A Cytocon™ Sorter Chip was mounted on a fluorescence microscope (Olympus IX-50) equipped with a fluorescein filter set set (BP510-550, DM570, BA590) and connected to the Cytocon™ Fluidics System and the Cytocon™ Chip Driver.
- The cell samples were mixed to a concentration of $2.5 \cdot 10^6$ *Jurkat* cells / ml and $1.8 \cdot 10^7$ red blood cells / ml. Samples of 0.3 μ l were injected into the Sorter Chip.
- The cells were separated based on differences in their size and dielectric properties by the switch electrode of the Sorter Chip using a frequency of 800 kHz. Under these conditions *Jurkat* cells were deflected and collected in fraction 1 whereas red blood cells could pass the switch electrode group and were collected in fraction 2.
- *Jurkat* cells and red blood cells were counted in both fractions to determine the enrichment and yield.

separation based on properties inherent in the cells

separation based on difference in size or presence / absence of a nucleus

no addition of a label required

easy to automate

separation of very small numbers of cells is possible

high yields for low – abundance target cells

very gentle conditions for live cells