



Isolation and enrichment of rare cells with an immunomagnetic cell selection system

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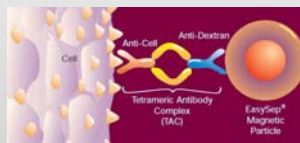
Introduction

Cells of biomedical interest are, despite their functional significance, often present in very small numbers. Therefore the analysis and isolation of previously inaccessible rare cells, e.g. peripheral haematopoietic stem cells or circulating tumor cells, require efficient, sensitive and specific procedures that work under physiological conditions. Furthermore the automation of laboratory processes is an important requirement for streamlining and standardizing technical procedures.

Methods

RoboSep® (StemCell Technologies Inc.) automates an immunomagnetic cell selection procedure (Easy Sep®) that uses specific antibodies and tiny magnetic nanoparticles in a column-free magnetic system. Cells can be targeted either for selection or depletion using monoclonal antibodies directed against cell surface antigens. These labeled cells are then crosslinked to magnetic dextran iron nanoparticles and separated in a specially designed ring magnet. Unlike larger microparticles these nanoparticles do not interfere with subsequent analysis (e.g. FACS). We used the RoboSep® System to separate rare cells from the vast majority of peripheral blood cells. Selected cell subpopulations were analyzed by immunohistochemistry, immunofluorescence and flow cytometry.

Schematic of Easy Sep® TACs
Magnetic labeling of cells



TACs are comprised of two mouse IgG1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG1 monoclonal antibody molecules. One mouse antibody recognizes the specific cell surface antigen while the other recognizes dextran on the magnetic particles.

(http://www.stemcell.com/technical/28960_RoboSep_v4.pdf)

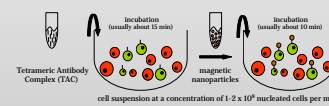


It is also possible to use a biotin-, PE-, APC- or FITC-conjugated, or any mouse IgG1 antibody directed against a cell surface antigen on your cell of interest.

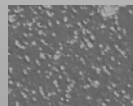
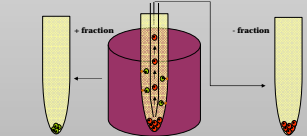
(http://www.stemcell.com/technical/28960_RoboSep_v4.pdf)

Schematic of a positive selection procedure:
Incubation and separation regiment may be varied for either optimizing purity or recovery.

A Labeling of cells



B Separation of labeled cells



cells in +fraction

Robotic arm: uses 1 ml tips to add reagents and 5 ml tips to add buffer and transfer or mix cell sample.



RoboSep®

Allows positive or negative selection of virtually any cell type from any single cell suspension or species. Isolates cells directly from any sample source including whole blood. Isolated cells are immediately available for flow cytometry, functional studies, or downstream analysis.



Fits into a standard biocontainment hood for sterile operation.

Technical specifications :

- Dimensions:**
 - Height with lid: 56 cm
 - Width: 71 cm
 - Depth: 39 cm
 - Weight: 26 kg
- Power Requirements:**
 - 50/60 Hz, AC 100-240V
 - Connections: RJ-45 10/100 Ethernet port, 2 USB ports
- Conditions for Operation:**
 - Temperature: 10-30°C (50-86°F). RoboSep® is not specified for use in a cold room (4°C, 39°F)
 - Humidity 20-85% (non-condensing)



Carousel: each quadrant contains a magnet, sample, tip rack and reagents. This enables separation of up to 4 samples in parallel.



Intuitive touch screen operation: after selecting the relevant protocol, a list of required reagents and resources is displayed. The instrument calculates the reagent addition volume based on sample volume.



Capacity:
Positive selection: up to 2x10⁹ each sample
Negative selection: 1x10⁹ each sample
Sample volume: from 250 µl to 8.5 ml per sample



Tip racks: each disposable rack contains sterile tips to process a single sample.

http://www.stemcell.com/product_catalog/robosepinstrument.aspx

Results:

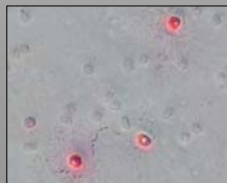
Initially CD90-positive cells were enriched from the leucocyte-fraction of peripheral blood. These cell population was further characterized.

It is possible to isolate high yields of vital CD90-targeted cells with the RoboSep® System.

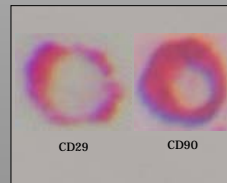
Conclusions:

The magnet-assisted cell sorting system RoboSep® is a suitable device for reliable enrichment of rare cell populations.

Immunohistochemistry (modified) ABC-method

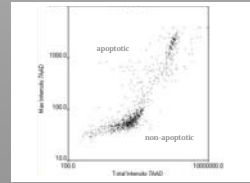


CD90-positivity is detectable by a biotinylated secondary antibody which recognizes the CD90-antibody bound during separation.

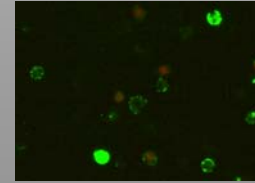


CD29 is detected using a biotinylated primary antibody to avoid a parallel recognition of the antibody used for separation (CD90).

Immunofluorescence

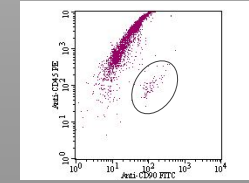


The cell vitality is detected by staining cells with 7AAD, which labels the nucleus of apoptotic cells (red).

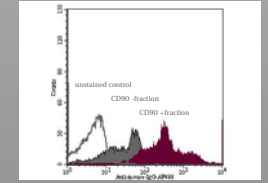


CD90-positivity is detected with a fluorescence-scanning-cytometer (Scan®) by labeling targeted cells with a fluorescence (AF488, green)-labeled secondary antibody which recognizes the CD90-antibody bound during separation. The nucleus of apoptotic cells is stained with 7AAD (red).

Flow cytometry



The use of labeled antibodies (e.g. CD90-FITC) for separation allows the analysis of targeted cells in comparison to other markers, e.g. CD45.



CD90-positivity is detected by flow cytometry, using a fluorescence (AF488, green)-labeled secondary antibody which recognizes the CD90-antibody bound during separation.