Cell Separation

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Basic Methods

- Separation according to density (and size):
  - Gradient centrifugation
- Separation according to adhesion
  - Adhesion to surfaces
  - Adhesion to sheep erythrocytes: E-rosette formation
- Separation according to surface markers
  - Panning
  - Dynal beads
  - MACS
  - FACS

Differential Centrifugation Separation according to terminal velocity of particles

- Stoke's law
- $v_t = 2R^2(p_s-p)a/(9\mu)$
- v_t is the terminal velocity of the particle,
  - R radius of the particle
  - a centrifugal acceleration of the centrifuge
  - μ the viscosity of the medium,
  - p_s density of the particle
  - p density of the medium.

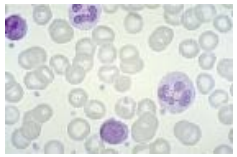
Density gradient centrifugation: separation according to density alone

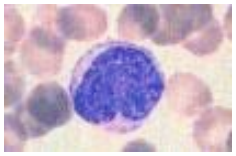
Example:

- Separation of MNC via Ficoll-Paque: Density of MNC (lymphocytes and monocytes) is lower than Ficoll-Paque and of erythrocytes and PMNL is higher.
- Ficoll-Paque: density 1.077 g/ml
  - Ficoll 400: a neutral, highly branched, hydrophilic polymer of sucrose which dissolves readily in aqueous solution.
- Recovery of MNC: 60 %, purity 95 %.

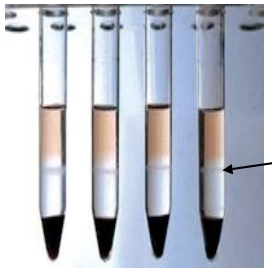
Blood smear

- Lymphocytes, PMNL, Monocyte



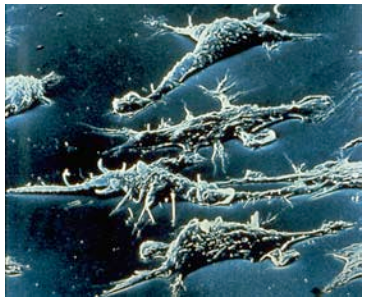


Isolation of human lymphocytes via Ficoll-Paque



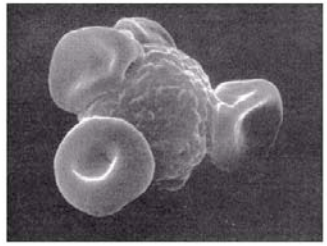
PMNL= polymorphonuclear leukocytes (Granulocytes)
MNC=mononuclear cells (lymphocytes and monocytes)

Separation of macrophages from MNC via plastic adhesion

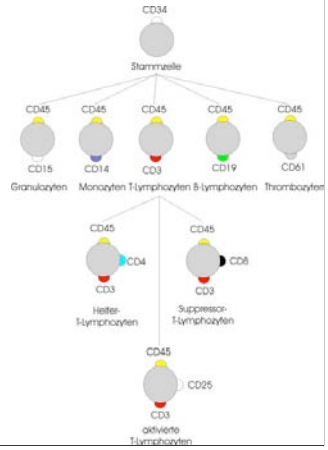


Isolation of T cells by rosette formation with sheep erythrocytes

Mediated by CD2 (T cell) and CD58 (erythrocyte) interaction

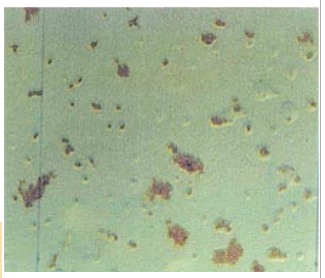


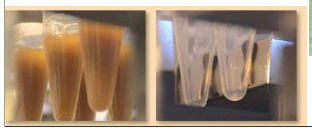
CD: Cluster of differentiation



Dynal beads

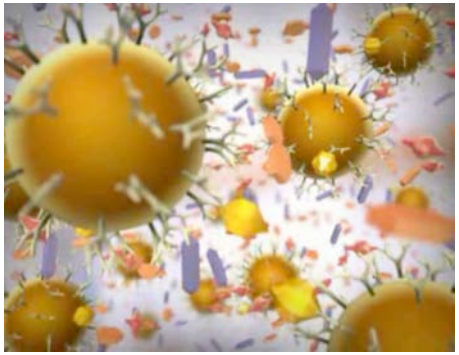
Supramagnetic beads
Coated with antibodies or
Other relevant ligands for
separation of cells or other
biological materials or
molecules





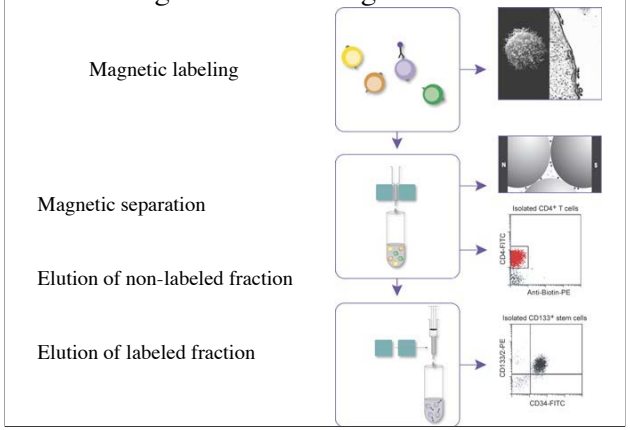
When added to a heterogeneous suspension, the Dynabeads will bind to the desired target (cells, nucleic acids, proteins or other biomolecules). This interaction is based on the specific affinity of the ligand on the surface of the Dynabeads. The resulting target-bead complex can be removed from the suspension using a magnet (Dynal MPC). Bead-bound complexes are drawn to the side of the tube facing the magnet, and the supernatant is removed with a pipette.

Dynal beads



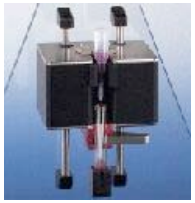
•The beads are superparamagnetic: that is, they exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field•The polymer shell of the beads/particles protects your target from toxic exposure to iron•The true uniformity (CV<3%) of size (appr. 2 µm), shape and surface area provides optimal accessibility and reaction kinetics, allowing for rapid and efficient binding•The true spherical shape and defined surface chemistry minimise chemical agglutination and non-specific binding•The specific characteristics of the many available bead types facilitate magnetic separation of a wide variety of targets•Unique batch-to-batch reproducibility (typically within 5%) secures the reproducibility and quality of your results•Separation is gentle and no column or centrifugations are necessary

MACS=magnetic cell sorting



MACS technology has become a standard method for cell separation by consistently providing high quality results. Numerous publications have demonstrated its use, in many different application fields. From lab bench to the clinic, from small to large scale, from frequent cells to rare cells and sophisticated subsets, from human and mouse cells to any other species, MACS Technology provides the tools that enable high quality cell separations in every lab. MACS Technology is based on the use of MACS MicroBeads, MACS Columns and MACS Separators. MACS MicroBeads are superparamagnetic particles that are coupled to highly specific monoclonal antibodies. They are used to magnetically label the target cell population. They are approximately 50 nm in size - not visible with light microscopy -, biodegradable, and gentle on cells.As the MicroBeads are extremely small, the use of a high-gradient magnetic field is required to retain the labeled cells. The MACS Column Technology is specifically designed to generate this strong magnetic field while maintaining optimal cell viability and function. By using a MACS Column with a coated, cell-friendly matrix placed in a permanent magnet, the MACS Separator, the magnetic force is now sufficient to retain the target cells labeled with a minimum of MicroBeads. By simply rinsing the column with buffer, all the unlabeled cells are washed out thoroughly, without affecting the labeled or unlabeled cell fractions, thus ensuring optimal recovery. By removing the column from the magnet, the labeled fraction can be obtained. With MACS Technology both the labeled and the unlabeled fraction are now highly pure, and an optimal recovery of the cells is guaranteed. The entire procedure can be done in less than 30 minutes, and the cells can immediately be used for further experiments.

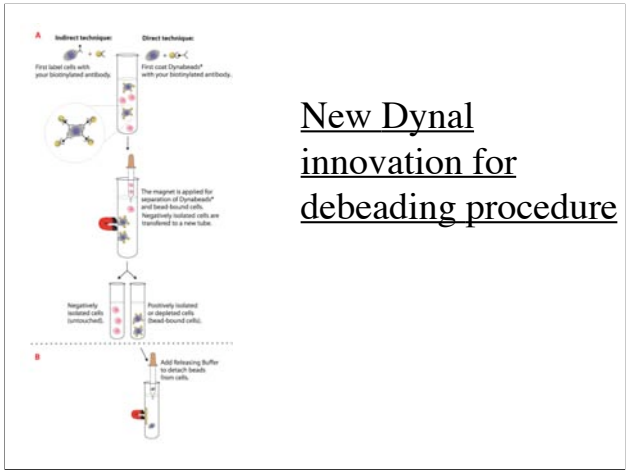
MACS separation unit



Dynal beads versus MACS

	Dynal beads	MACS
Size	1-3 µm	50 nm
Handling	Simple	More complex protocols
Price	cheap	More expensive
Final fate of beads	Must be removed by detachment step	Can remain on the cell
Negative selection according to single marker	Method of choice	Often to demanding technology
Negative selection according to multiple markers	difficult	Method of choice
Positive selection	Only for specific cell types	Method of choice

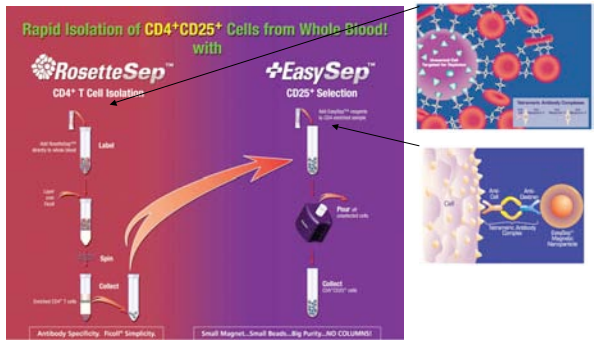
MACS and Dynal beads are both used for selection of specific cell subsets according to their expression of certain surface markers. MACS requires more expensive equipment and the procedure is longer and more demanding. An advantage of MACS is, however, that the extremely small MACS magnetic beads (size of a virus) can remain attached to the cell after selection, as they have so far not been found to have any detrimental effects on cell growth or functions. MACS technology is therefore preferentially used for postive or for complex negative selection according to several markers, e.g. depletion of memory T helper cells (CD45RA+ and CD4+). Dynal beads are the method of choice for negative selection according to a single surface marker (e.g. T-cell depletion with anti-CD3 coated beads).



New Dynal innovation for debeading procedure

The principle of cell isolation using Dynabeads® Biotin Binder (A) and CELLection™ Biotin Binder (A+B). Isolated target cells can only be detached from beads wih CELLection™ Biotin Binder.

StemCell Technologies



What is RosetteSep®? RosetteSep® is a rapid and easy cell separation procedure for the isolation of highly purified cells directly from whole blood. Utilizing *StemCell Technologies'* patented antibody-based TAC (tetrameric antibody complex)® technology, RosetteSep® turns a simple buoyant density centrifugation (e.g. Ficoll® step) into a specific, antibody-mediated cell enrichment system. Cell enrichment with RosetteSep® simply involves a brief incubation of the sample with the antibody-based enrichment cocktail at room temperature, followed by a standard buoyant density separation. The RosetteSep® antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes.. This increases the density of the unwanted (rosetted) cells, such that they pellet along with the free RBCs when centrifuged over a buoyant density medium such as Ficoll-Paque®. Desired cells are never labeled with antibody and are easily collected as a highly enriched population at the interface between the plasma and the buoyant density medium . Specialized equipment, such as a magnet, is not required and the enriched cells are ready for future use.

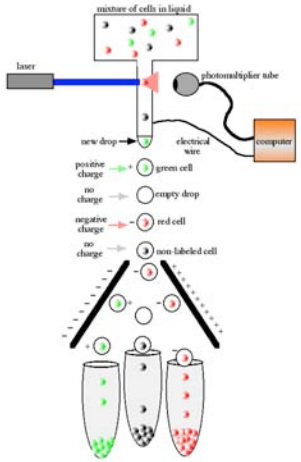
Human CD4^{+}CD25$^{+bright}$ T cells can be isolated quickly and easily directly from whole human blood! CD4^{+} T cells are first enriched using RosetteSep®, an immunodensity centrifugation procedure. The RosetteSep®® reagent is added directly to whole blood, followed by a standard Ficoll® centrifugation step. The CD4^{+} cells are collected from the Ficoll:plasma interface, while the CD4^{-} cells are pelleted with the red blood cells. CD25$^{+bright}$ cells are then selected from the CD4^{+} T cell-enriched sample using EasySep®. EasySep® is a simple, column-free magnetic selection procedure that uses FACS-compatible magnetic nanoparticles.®In a standard FACS tube, the EasySep® reagents are added to the cells, the tube is placed into the handheld EasySep® magnet, and the unselected (CD4^{+}CD25$^{-/+dim}$)cells are poured off, leaving the selected (CD4^{+}CD25$^{+bright}$) cells in the tube and ready for further study. With these two simple methods, highly enriched CD4^{+}CD25$^{+bright}$ T cells are obtained in less than two hours!

Fluorescence-activated cell sorting: FACS

•Technologically demanding and extremely expensive
•Even with high-spead sorting only a few mio cells can be sorted per day
•Reserved for complex sorts according to several parameters

In multicellular organisms, all the cells are identical in their DNA but the proteins vary tremendously. Therefore, it would be very useful if we could separate cells that are phenotypically different from each other. In addition, it would be great to know how many cells expressed proteins of interest, and how much of this protein they expressed. Fluorescence Activated Cell Sorting (**FACS**) is a method that can accomplish all these goals.

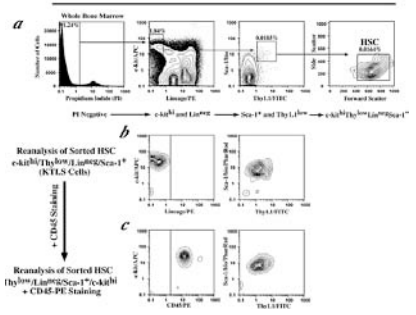
FACS machine diagram



The process begins by placing the cells into a flask and forcing the cells to enter a small nozzle one at a time. The cells travel down the nozzle which is vibrated at an optimal frequency to produce drops at fixed distance from the nozzle. As the cells flow down the stream of liquid, they are scanned by a laser (blue light in figure). Some of the laser light is scattered (red cone emanating from the red cell) by the cells and this is used to count the cells. This scattered light can also be used to measure the size of the cells.If you wanted to separate a subpopulation of cells, you could do so by tagging those of interest with an antibody linked to a fluorescent dye. The antibody is bound to a protein that is uniquely expressed in the cells you want to separate. The laser light excites the dye which emits a color of light that is detected by the photomultiplier tube, or light detector. By collecting the information from the light (scatter and fluorescence) a computer can determine which cells are to be separated and collected.

he final step is sorting the cells which is accomplished by electrical charge. The computer determines how the cells will be sorted before the drop forms at the end of the stream. As the drop forms, an electrical charge is applied to the stream and the newly formed drop will form with a charge. This charged drop is then deflected left or right by charged electrodes and into waiting sample tubes. Drops that contain no cells are sent into the waste tube. The end result is tubes with pure subpopulations of cells. The number of cells is each tube is known and the level of fluorescence is also recorded for each cell. This system does not work if the cells are excited and measured in a cuvette, like in the pure flow cytometry analysers but requires a ‚flow in air' system.

Example: Sorting of hematopoietic stem cells



An example is the isolation of CD45-positive mouse HSCs.

a, Phenotypic analysis of adult bone marrow cells and the restricted gates used to sort HSCs.

b, Analysis of the sorted KTLS HSCs.

c, CD45 analysis of the sorted HSCs. HSCs were sorted a second time for CD45 positivity.