

Topic : Flow Cytometry
[Cell separation by Flow Cytometry]

Prof. Dr. V. Kumar
Dept of Zoology.

Flow Cytometry is a technique in which optical system is used to identify and collect different types of cells, cell organelles, metaphase chromosomes or cell macromolecules.

The instrument used for cell sorting is called ~~the~~ Fluorescence-activated cell sorter or cytometer. This is a microfluorometer.

In this technique, a fluorescent dye is linked to make at highly specific staining reagent. Fluorescent dyes use Fluorescein and Rhabdomine.

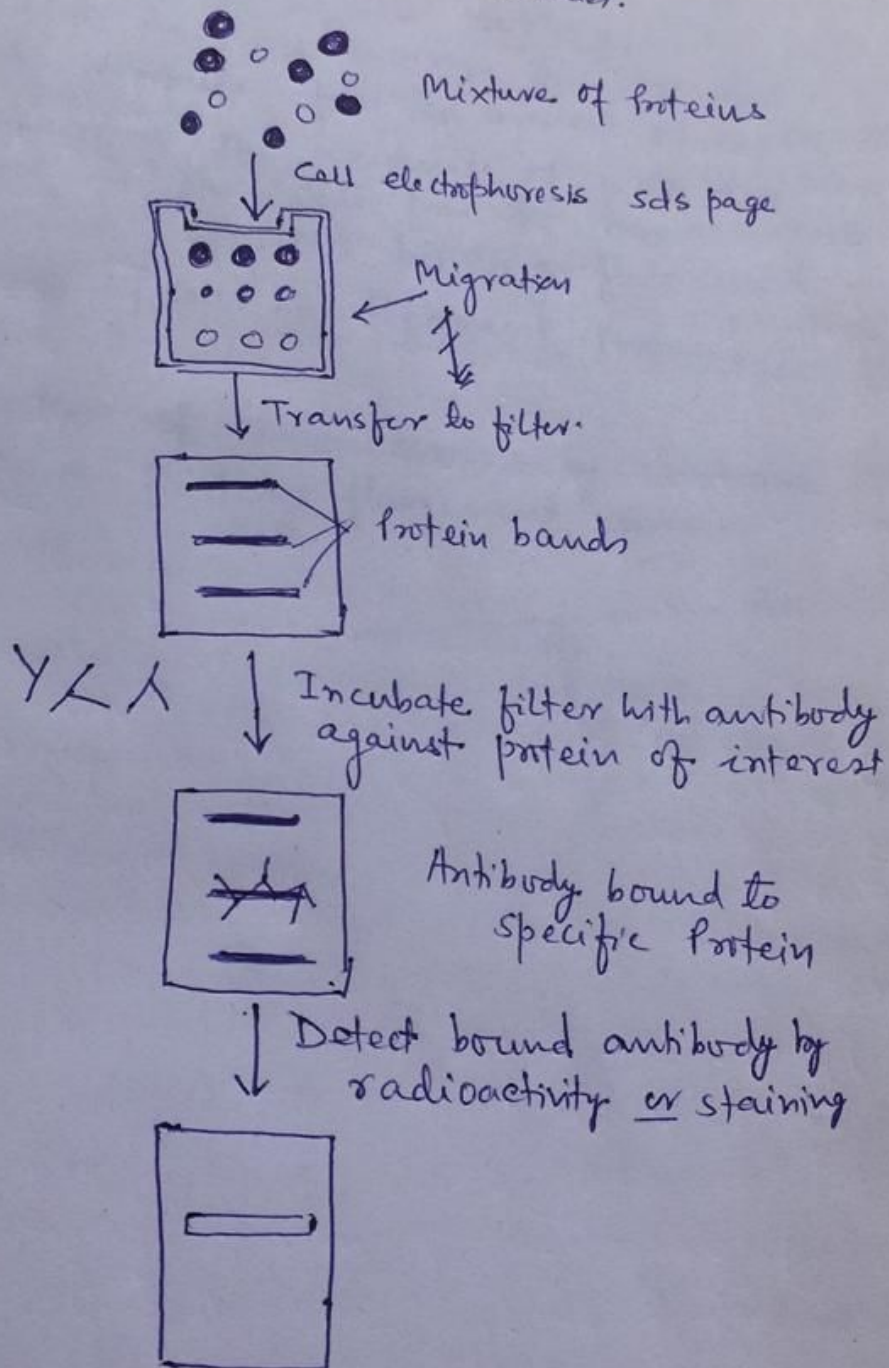
Fluorescein emits green light when excited with blue light and Rhabdomine emits red light when excited with green yellow light.

When specific cells are to be sorted out the antibody that is specific to the antigen present on the surface of these cells is chemically conjugated with a fluorescent dye. The cells bearing this particular antigen binds with the antibody.

Inside the cell-sorter, these cells with antibody-fluorescent dye complex are exposed to the correct wavelength of light, delivered by a Laser beam. These emits fluorescent light and a flowing stream of tiny droplets of liquid from the cell suspension medium is formed. Each droplets has at least one fluorescent cell. The fluorescence of each droplets is measured by detector.

The droplets having fluorescent cells are given negative electric charge. When stream passes between two electrically charged plates, the droplets with negative charge get separated. The FACS

②
Selects these charged cells with specific marker in the form of fluorescent antibodies.



Process of sorting out proteins from a protein mixture by flow cytometry

Other uses of Flow Cytometry

(3)

- In addition to cell sorting, this technique is used in measuring the size of the cells in the droplets from the amount of scattered light.
- Measuring the amount of DNA and RNA present in the cell from the amount of fluorescence of DNA-binding fluorescent dyes.
- Sorting of cells in different phases of the cell cycle.
- Isolation of chromosomes by staining them with DNA-binding fluorescent stain.

Nigay Br.
20.4.2020.