ELISPOT Assay:

- It is a variation of sandwich ELISA method.
- It stands for Enzyme linked Immunosorbent Spot assay.

Uses of ELISPOT assay:

- It is used to identify the cells in a population that are secreting a specific molecule such as a cytokine or a hormone etc.
- It is also used to identify antigen specific T or B-cells in a population of cells after antigen specific stimulation.
- It is used to determine the changes in the frequency of antigen specific cells after a treatment course.

Process outline for ELISPOT assay:

The process given below is described for the detection of the interferon-γ (IFNγ) secretion by NKT cells. The NKT cells are a group of cells that express cell surface markers that are representative of the T-cells as well as NK cells.

These cells express following markers:

- Semi invariant β-chain of the TCR.
- Invariant α-chain of the TCR.
- NK 1.1 which is also known as KLRB1 or CD161.

These cells produce various cytokines upon antigen encounter in the body and one of these is interferon-γ.

- Assay plates are coated with anti- IFNγ antibody. This is called as capture antibody as it will bind to the IFNγ secreted by individual cells and capture it before it can diffuse into the culture.
- Now we add a suspension of the cells to be assayed.
- Cells are now incubated with an appropriate stimulating agent such as phorbol myristoyp acetate (PMA) and ionomycin.
- The cells have settled onto the surface of the plates and any IFNγ secreted by the stimulated cells will bind to the capture antibodies present in the well.
- This creates a ring of IFNγ – antibody complexes around each IFNγ producing cell.
- The plate is now washed to remove cells and we add an enzyme labelled antibody. This antibody is called as detection antibody as it binds to the IFNγ but on a different epitope than the one bound by the capture antibody.
- Now, unbound detection antibodies are removed by washing and we add a substrate which is specific for the enzyme label present on the detection antibody.
- One of the most common substrates for alkaline phosphatase labelled detection antibodies is BCIP i.e. 5-bromo-4-chloro-3-indolyl phosphate. It is also known as NBT or nitro blue tetrazolium.
- On reaction with enzyme, this substrate is converted into an insoluble brown-black coloured precipitate.
- Position of this precipitate marks the spots where detection antibody was bound.
- Each spot represents the site of a cell that secreted IFNγ.
- The number of spots are counted by placing the reaction well under the dissecting microscope or by using handheld magnifying lenses.

- After this we compare the number of spots to the number of total cells added in the reaction well. This tells us the fraction of population which is secreting the given cytokine or molecule which in this case is IFNγ.