RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY

Course: B. Tech Biotechnology
Sub Code: BBT-515

Semester: 5th
Sub Name: Plant Biotechnology

LECTURE 5

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MICRO DROPLET TECHNIQUE

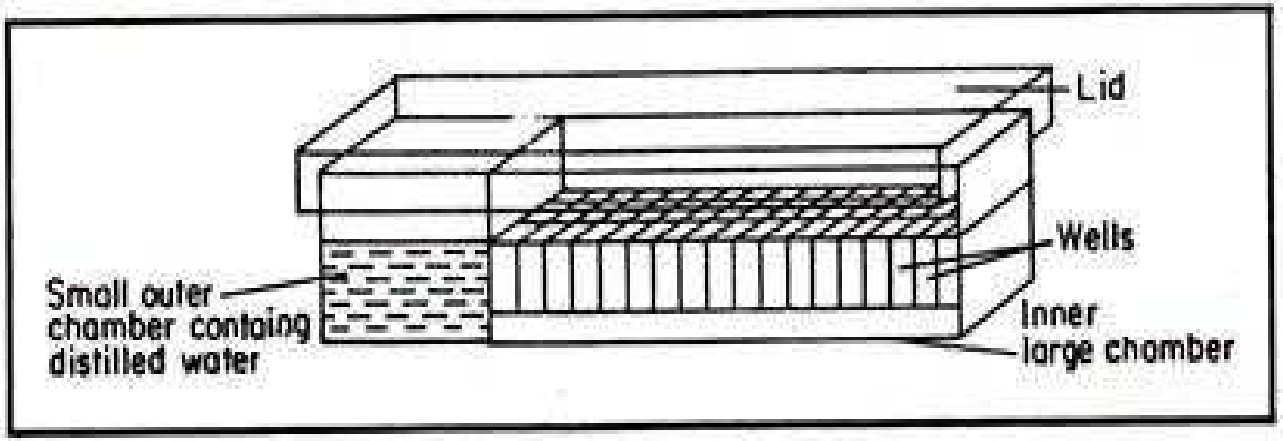
- The technique requires a specially designed cuprak dishes which have smaller outer chamber and larger inner chamber.

- The inner chamber carries numerous numbered wells, each with a capacity for 0.25 to 25 µl droplet of nutrient medium.

- Individual cell is transferred to each well of the chamber along with conditioned medium.
- The outer chamber is filled with sterile water to maintain humidity inside the dish.

- After covering with lid, the dish is sealed with parafilm and maintained at optimal light and temperature conditions.

- When cell group develops it is transferred to medium to form callus



❒ Fig 9.5

Diagrammatic view of Cuprak dish used for the microdroplet technique of single cell culture

https://www.biologydiscussion.com/plant-tissues/single-cell-culture/single-cell-culture-5-methods-with-diagram-plant-tissue-culture/14699

CULTURE CELL VIABILITY TEST

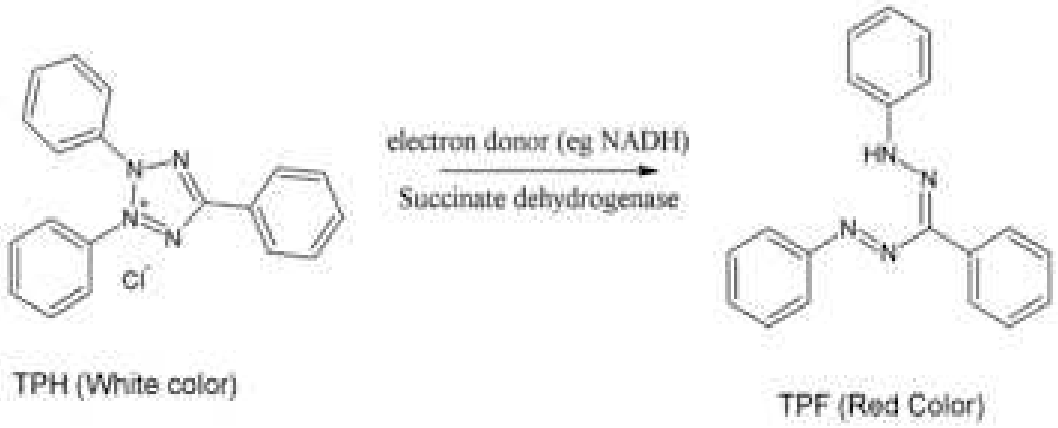
Phase contrast microscopy:

Using phase contrast microscopy, the cytoplastic streaming and the presence of healthy nucleus will be observed.

Thus the viable cells are counted for the given volume of culture.

Reduction of tetrazolium salts:

When the cell cultures are incubated with 2,3,5 triphenyl tetrazolium chloride (TTC), the viable cells converts theTTC into a red colored substance known as 1,3,5 triphenyl Formazan, which is estimated spectrophotometrically.



TPH (White color)

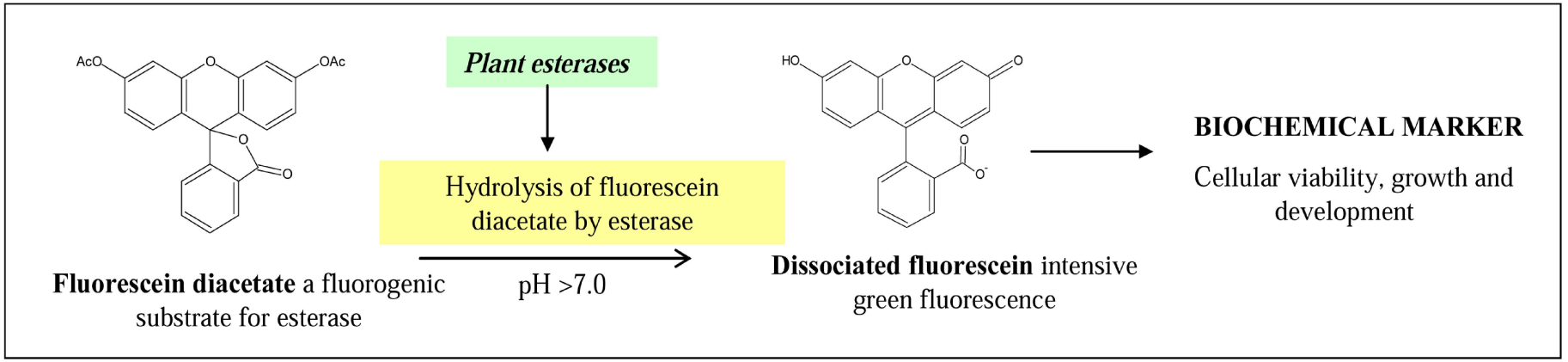
TPF (Red Color)

Fluorescein diacetate (FDA) method:

Cell cultures are incubated with 0.5% FDA for 5 min.

FDA being non polar and non fluorescing, enters the cells and is cleaved by esterase activity in the living cell resulting into polar Fluorescein.

Since, fluorescein is not freely permeable across the plasma membrane, it accumulates mainly in the cytoplasm of intact cells, thus those cells exhibit green colour fluorescence.



Evans Blue method:

Cell cultures are incubated with 0.025% Evans blue for 5 min.

Evans blue dye has been used as a viability assay on the basis of its penetration into non-viable cells i.e dead and damaged cells.

The dead or damaged cell takes the blue colour.

QUIZ