## **EXERCISE - 5**

## • Preparation of chick embryo fibroblast primary cell culture

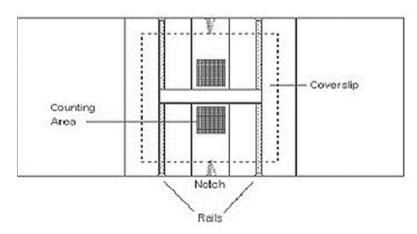
## <u>REQUIREMENT</u> –

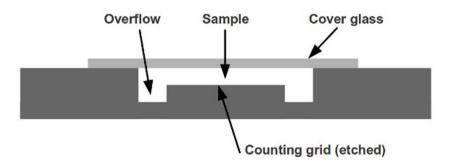
- 10 days old embryonated egg,
- egg candler,
- tincture iodine,
- incubator,
- forceps,
- petridish,
- BSS Balanced Salt Solution-Hank's,
- two scissors straight and curved,
- Beaker,
- waterbath,
- trypsin,
- trypsinizing flask,

- magnetic bar,
- magnetic stirrer,
- centrifuge tube,
- funnel,
- MEM (Minimum essential medium)
- FCS (Fetal calf serum)
- growth medium (MEM with 5% FCS),
- maintenance medium (MEM with 2% FCS)
- cell culture flask
- Inverted microscope
- Neubauer's cell counting chamber

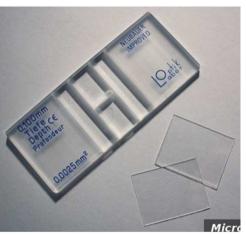












## PROCEDURE -

Collect 10 days old fertile eggs

Select proper one by candling (only well developed, active embryos with good blood supply)

Apply tincture iodine on the entire surface of the egg

Keep the egg's air sac end uppermost

Break the egg shell at the air sac with the help of sterile forceps

Remove the shell, the shell membrane and the chorioallantoic membrane

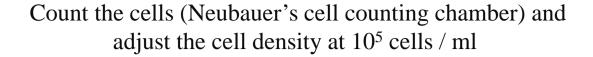
Take out the embryo, catching it by the beak using the curved forceps and place it in a petridish containing BSS

Wash the embryo several times with BSS

Remove the head and limbs using a pair of scissors and a forceps. Then remove the internal organ by tearing the abdominal wall with the scissors.

Transfer the embryo to a beaker containing BSS and wash it several times with BSS

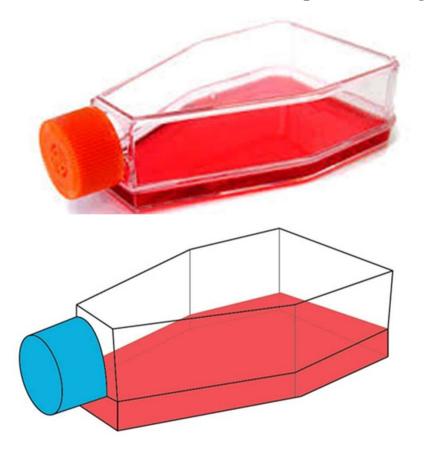
Mince the embryo with the help of a pair of curved scissors Wash the minced tissue with BSS several times by pouring fresh BSS Give a final washing with trypsin solution warmed before at 37°C Transfer the tissue pieces to a trypsinizing flask containing a teflon coated magnetic bar and pour sufficient amount of trypsin Place the trypsinizing flask on a magnetic stirrer and run it at a moderate speed (15 minutes) Allow the tissue fragments to settle, and then discard the supernatant Pour fresh trypsin solution and continue trypsinization for 30 minutes 2-3 times till almost all the tissue fragments are digested Centrifuge the cell suspension at 1000 rpm for 10 min Wash two times with BSS and finally with the growth medium Suspend the cells in 100 ml growth medium

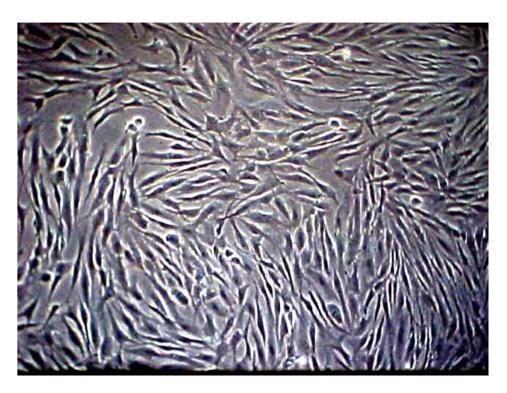


Distribute the cells in cell culture vessels

Incubate at 37°C in the incubator

Confluent monolayer forms in about 3-5 days. (If required, change the medium on the third day)





SN	Components	Use(s)
	BSS – Balanced Salt Solution-Hank's	<ul> <li>- A solution made to a physiological pH and isotonic salt concentration</li> <li>- Provide the cells with water and inorganic ions, while maintaining a physiological pH and osmotic pressure</li> <li>- Glucose is added as an energy source and phenol red is used as a pH indicator</li> </ul>
	Trypsin	-Used to dissociate dissected tissue into cells -Used to resuspend cells adherent to the cell culture flask
	Trypsinizing flask	<ul><li>-Used for converting homogenous tissue samples into cell suspension by digestion of connective tissue proteins.</li><li>- Deep baffles enhance vigorous agitation</li></ul>
	Magnetic stirrer	-
	Magnetic bar	
	MEM (Minimum essential medium)	-Used for growth and maintenance of cells of cell culture - Provides physical conditions such as pH, O2, CO2, osmotic pressure and nutrition to the cells in the form of chemical substances such as amino acids, carbohydrates and vitamins.
	FCS (Fetal calf serum)	- Used for protein requirement of the cell growth in cell culture.

SN	Components	Use(s)
	Growth medium (MEM with 2% FCS)	- Medium used for growth of cells in cell culture
	Maintenance medium (MEM with 5% FCS)	- Medium used for maintenance of cells in cell culture
	Cell culture flask	
	Neubauer's cell counting chamber (Haemocytometer)	