

## **GENERAL MAINTENANCE INSTRUCTIONS FOR IMAGINAL DISC CELL LINES (Nusse lab)**

Cells are passaged every 3-4 days from confluent T25 flasks (about 5 x 10<sup>7</sup> cells): split roughly 1/5 to 1/10. Never allow cultures to become too dense for they will die rapidly! If 95% are dead the day after you split them you have probably let them grow too dense. For the same reason, always use subconfluent cells for biochemistry or RNA isolations. These cells are tightly adherent, especially when they have been passaged recently. Wash the cells with 3ml of trypsin, prior to trypsinization. Trypsinize for about 2 min at room temperature. Tap the flasks real hard to detach cells, immediately add 5 ml of medium to inactivate the trypsin. Spin down cells at 1200rpm in a table top centrifuge and resuspend to required density. Always use a fresh flask when transferring cells. Cells can be frozen in complete M3 medium + 10% FCS and 10% DMSO; again use subconfluent cultures.

## **MAKING FLY EXTRACT FOR WING DISC CELL MAINTENANCE**

(Cullen and Milner, *TISSUE AND CELL*, 1991 23(1):29-39.)

Start with a collection of about 30g of healthy flies. 200 flies weigh 0.22g. Use 1.5ml M3 medium per 0.22g; to make 200ml homogenate, use 30g of flies.

Place flies in freezer for 20 min.

Place 30g of frozen flies and 200ml of medium into a blender. Puree" in blender approximately 2-3 minutes.

Spin mush at 2600 rpm in a table top centrifuge.

Remove supernatant (leaving exoskeletons and eye pigment) and transfer to a new tube. Remove oily layer on top. Heat inactivate in 60 C waterbath for 30 min. You will see a precipitate form. Centrifuge at 2600 rpm for an hour. Remove supernatant and sterilize through a 0.22 um filter. Store in 12.5 ml aliquots (2.5% final in 500ml) and keep at -20 C.

## **REAGENTS**

The complete M3 medium has the following additives.

2% FCS

**2.5% fly extract**

**0.125 iu/ml insulin**

**1/2 x penstrep (100 x bottle = 5000 ug/ml)**

**Making insulin stock.**

**Insulin (Sigma, I-6634)**

**Dissolve 10mg (25iu) in 20mls of M3 medium to make a 100 x stock.**

**Dissolve insulin in 0.5ml of 0.01n HCl.**

**Add medium to 20ml**

**Filter through 0.22uM filter**

**Make fresh stock everytime.**

**Trypsin**

**Gibco BRL Trypsin - EDTA .05% Catalog #25300-054**

**PBS**

**Sigma #D5927**

**M3 Medium**

**Shields and Sangs M3 medium, Sigma (#S3652)**

**FCS (heat inactivated tested for insect culture), Sigma (#F3018)**

## **TRANSFECTIONS**

**Imaginal wing disc cell lines can be transfected using the conventional Ca<sup>++</sup> phosphate coprecipitation method. Cells should be split 1:4 the day prior to the transfection from a subconfluent T75 flask into 10cm dishes.**

**Cells are left with the DNA coprecipitate overnight. The next day the precipitates should be washed away with PBS, trypsinized and transferred to a clean dish. The cells should be allowed to recover one day before drug selection is started. Transients also work well in these cells.**

**For drug selection 250 ug/ml hygromycin seems to give good results although early on during the selection some drug resistant colonies do appear. Colonies should appear in about 14 days. When the colonies consist of a few hundred cells they can be scraped off the dish with a sterile yellow tip and transferred to a 12 well dish. Usually after another 2 weeks or so these can be transferred to a T25 flask.**

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