Written by David J. Niedzwiecki **11/13/2010 4:02:15 PM**

Cell splitting and preparation protocol for CHO cells.

F12 media
PBS
Tripson
CHO cell line at 80-90% confluence
5 & 10 mL pipette
Orange capped cell flasks
Autoclaved glass pasture pipettes

IMPORTANT: everything that goes into the fume hood must be sterile. Gloves must be worn at all times and must be sprayed with 70% ethanol before touching anything in the hood. Pollution of the sample will result in the destruction of the cell line, necessitating its regrowth and resulting in a 2 week delay of further experiments.

Spray the base of the fume hood with 70% ethanol and wipe down.
Pre-heat F12 media, Tripson and PBS in the 37 degree Celsius bath for at least 10 minutes.

Open incubator and check cell line for 80-90% confluence. (Note: at more than 95% cells will start to die, at less than 80% , the new cell lines will take a long time to reach high confluence.)

Turn on vacuum pump and attach a glass pasture pipette to the vacuum tube.
Remove cell media from the old cell line flask (orange cap).
Wash the flask with 5-6 mL of PBS buffer (equilibrates pH and washes dead cells away)
Gently swish the PBS in the old flask.
Use another glass pasture pipette to evacuate the PBS.

Add 2 mL of Tripson to the flask
Seal the flask and swish around the Tripson
Place in the incubator for 3-4 minutes.

Label a new flask
Add 14 mL of F12 media to the new flask (use 10 mL syringe)

Remove old flask from incubator and check to see if CHO cells have disassociated from the surface. (Shake a little to see if they are easily removed)
If cells still adhere to the surface of the flask, place in incubator for another 2 minutes.

Once the cells are floating in the Tripson, wipe down the flask with 70% ethanol and put it in the hood.

Add 3mL of F12 media to the old flask to stop the action of the Tripson.

Pipette up and down, coating the surface of the old flask at least 10 times.

A 1:8 ratio (old flask solution: new flask media--by volume) of the resulting solution should now be added to the new flask. The 1:8 ratio should be used if cells were at 90% confluence originally; this ratio should be higher (example 1:6) if cells had lower expression.

If preparing a Petri dish for patch clamping experiments, the same 1:8 ration should be used. The F12 media may be added before or after the CHO cells.

Incubate the new flask (and Petri dish) when done.

This process should be repeated every 2 days.