Maintenance of HFE-producing CHO cells

The procedure that I followed to harvest the supernatant from the HFE-producing cells is basically the standard operating procedure followed in the lab for harvesting CHO cells producing soluble proteins.

There are ten HFE-related cells lines that either Christina or I produced--mHFE, hWt, H41D,S43C, W81A, V78A, W141A, Double his mut, tetra his mut. All of these cell-lines can be found in the liquid nitrogen storage (refer to the Lab Cell-lines binder for the exact location). There should be multiple vials for each of these cell-lines.

Thawing the cells

1) Warm up a bottle of glutamine-free alpha-Minimum Essential Media (α-MEM) in the tissue culture (TC) water bath for at least 10 minutes.
2) Thaw a 25-ml aliquot of decomplemented dialyzed fetal calf serum (dFCS). Serum must be dialyzed in order to remove glutamine.
3) Thaw a 5-ml aliquot of penn/strep (P/S).
4) Thaw a 550 µl aliqout of 100 mM methionine sulfoximine (MSX).
5) Wipe hood with 70% ETOH, then irradiate with UV light for at least 10 minutes.
6) Take all of the above solutions out of the water bath and dry the outside of these containers.
7) Turn the hood UV light off.
8) Wipe with 70% ETOH all of the containers before placing them inside the hood.
9) Inside the hood, add the 5 ml of P/S, the 25 ml of dFCS, and the 550 µl of MSX to the 500 ml of α-MEM, then mix. This solution will be refered to as α-MEM complete.
10) Get the desired frozen vial containing the corresponding cell line. Wipe the outside of the vial with 70% ETOH and place the vial in the hood.
11) Get a sterile 10-cm tissue culture plate (Falcon 3003) and place it inside the hood.
12) Thaw the cells by rubbing it against your gloved-hands.
13) Once the cells are thawed, dump them into the 10-cm plate, then add 15 ml of α-MEM complete.
14) Grow the cells overnight in the TC incubator.
15) On the following day:
    a) Warm up the media for 15 minutes. Wipe with ETOH and place in hood.
    b) Remove the cells from the incubator, take a look at them on the microscope--they should be already attached to the plate--then put them in the hood.
    c) Remove the media from the plate using a sterile pasteur pipet connected to the vacuum pump. The media is changed the day after thawing the cells to get rid of the DMSO used for freezing the cells.
    d) Add 10 ml of α-MEM complete. Put the plate back in the incubator.
16) Check the plate everyday until they reach confluency, changing the media every other day.
17) Once the cells reach confluency, they can be splitted into several plates. At this point I normally split the cells at a 1:5 ratio (i.e. one plate is splitted to five plates).

Spliting the cells

1) Warm up a bottle of Trypsin/EDTA solution.
2) Wash the cells at least twice with Sterile phosphate buffered saline (PBS) solution. To do this remove the media from the plate and add 3-5 ml of PBS, then swirl the plate to wash the cells. Remove the PBS and add another 3-5 ml of PBS, then swirl the plate. Remove the PBS.
3) Add about 0.5-1.0 ml of Trypsin/EDTA solution to the cells, making sure that all the cells on the plate are covered with this solution, and incubate for about three minutes, or until all the cells have detached from the plate.
4) Add 50 ml of complete media to the cells, then pipet up and down with a 25 ml pipet to evenly dirperse the cells. Aliquot 10 ml of the cell solution to each of five pre-labeled 10-cm TC plates. I like to remve the cells this way (i.e., adding the media to the trypsin EDTA solution) because I transfer more cells that way and because it is faster. However, there are at least two more ways of splitting the cells. Some people remove the Trypsin/EDTA solution, as well as some of the cells, then add the media. Others, add PBS to the cells after the cells are detached, but without removing the trypsin/EDTA solution, then centrifuge the cells and resuspend them in media. Any of these procedures works equally well.
5) Place all five plates into the TC incubator.
6) Check cells every other day for confluency, changing the media on those days.
7) When the cells reach confluency, repeat steps 1-4 with the following modifications:
    a) Instead of spliting the cells 1 to 5, do a 1 to 10 split (i.e. one plate split to 10 plates) to obtain 50 cell-containing plates.
8) Change the media every other day until the cells reach confluency. At that point harvest the cell supernant as follows:
    a) Transfer all the plates to the hood. Using a 25 ml disposable, sterile pipet collect the media from all the plates.
    b) Using the same pipet as in (a), add 10 ml of complete media to each of the plates.
    c) Put the plates back into the incubator.
9) Add Na-azide to the harvested supernatant and filter it through a 0.45 micron filter. Store the harvest in the cold room.

Notes:

1) Normally people in the lab maintain 50 plates of a particular cell line at a time. However, it is also possible to maintain ~100 plates instead. What I do is to repeat step (7) using five of the 50 plates that I am currently maintaining. About five days later I will have another 50 plates to harvest supernatant from.

2) The cells usually last about three to four weeks in culture. Therefore, a new set of 50 plates needs to be prepared about every two weeks.