Harvesting proteins from Chinese Hamster Ovary cells

Selection Media:

Custom alpha MEM from Irvine Scientific (NO L-glutamine!)
5% heat inactivated dialyzed FBS
5mL Penicillin/Streptomycin
500 uM MSX

1. Thaw cells from the Dewar in a $10cm^2$ dish with 10mL fresh selection media.
2. Once cells are confluent, split into a $15cm^2$ dish with 25mL media.
3. Once cells are confluent again, split into a $22.5cm^2$ flask or more $15cm^2$ dishes for harvesting*.
4. If harvests are for immediate protein purification, split into as many dishes or flasks as needed. If harvesting to maintain our lab stocks, split into 5 x 22.5 cm^2 flasks for gradual, long-term (several months to a year) collection.
5. Feed cells every 2-3 days with fresh selection media (i.e. every Monday, Wednesday and Friday of every week). Before collecting the old media, check for contamination first. If the cells are contaminated, quickly dispose of the dirty flask(s). In my experience, having one contaminated flask does not mean that the whole batch is contaminated. If you find more than one dirty flask, however, it may be best to just dispose of the current cells and thaw a new vial of fresh cells.
6. Collect harvests into 1-liter 0.22um filter units (with bottles attached for sterility). Once container is full of protein-rich harvest, add 0.05% sodium azide.
7. Store harvests in the cold room or at 4^0C.
8. Split cells every 10-12 days to keep them happy. Before disposing of any excess cells, make sure we have enough frozen stocks in the Dewar first (15 vials or more). You will recognize the importance of this once you realize how long it takes to get stably-transfected, high protein-expressing cell lines.

*A $15cm^2$ dish usually holds 25-30mL of media for comfortable handling while a $22.5cm^2$ flask usually holds about 50-60mL of media.

Created by Noreen Tiangco