Frozen Cells Instructions

NOTE: this protocol is written for a hybridoma cell line of average health and growth rate. Not all lines are the same in terms of their health or rate of growth. Adjustments must be made concerning both the volume and the rate at which media is added. Cultures tend to grow best when kept between $5X10^5$ and $1X10^6$ cells/ml. When the health of the culture is in doubt, do not add media. Be patient and wait for the cells to display healthy growth and appropriate cell density. Hybridoma lines are a mixture of both adhered and suspended cells. Bright and round cells, turbid medium, the presence of cell clusters, and a drop in the pH of the medium are all indicators of a healthy culture. Dark and shriveled cells, cell debris, and no increase in the number of live cells are indicators of poor health. Trypan blue can be used to determine the percentage of viable cells. If the cells appear to be worsening in health i.e. the number of dead cells is increasing, consider moving the entire culture to a smaller flask or well-plate until it is able to recover.

Thawing Cells

Thaw the cells in less than 2 minutes in a 37°C water bath. When the cells are just thawed, quickly transfer them to a tube containing 5ml of culture medium. Spin the cells at 170Xg for 5min. Carefully draw off and discard the medium. Transfer cells to a T-75 flask containing 10ml of complete culture medium.

Freezing Cells and Supernatant Preparation

Flasks should be placed in an incubator at 37°C with 5.0% CO_2. Make sure the caps are vented to keep the medium at the proper pH. Approximately 1-2 days later, when the cells appear healthy and are dividing and the cell concentration is between $5X10^5$ and $1X10^6$ cells/ml, add 10ml culture medium. Approximately 1-2 days later, split the culture evenly into two T-75 flasks and add 10mls of additional culture medium to each flask. Label one flask FREEZE and the other SUPERNATANT.

Freezing Cells (3 X 1ml aliquots)

Approximately 1-2 days later, add 20ml of culture medium to the FREEZE culture. Frozen cell aliquots can be made while the cells are in log phase growth and the density reaches approximately $5X10^5$ and $1X10^6$ cells/ml. Pellet the 40ml FREEZE culture by spinning at 170Xg rpm for 5 minutes. Carefully draw off and discard the supernatant, resuspend the cell pellet in culture medium with cryoprotectant (6% DMSO), and transfer to the suspension to cryovials. Place the cryovials in the vapor phase of liquid nitrogen and freeze at a concentration of approximately 1X107 cells/ml. Normally, three 1ml aliquots can be frozen from a 40ml culture.

Supernatant Preparation (200ml)

Approximately 1-2 days later, split the SUPERNATANT culture into 2 flasks and add 10ml of serum-free medium to each. Maintain the culture between $5X10^5$ and $1X10^6$ cells/ml by adding serum-free medium over the course of several days. Once each flask contains 50ml of culture and the density is approximately $1X10^6$ cells/ml, transfer the contents (100ml total) into a non-vented 490cm^2 roller bottle and add 100ml more of serum-free medium. The roller incubator should be set at 37°C and bottles should rotate at approximately 1rpm. If a roller bottle setup is not available, the antibody production phase can be done in multiple static flasks with approximately the same combined surface area as the roller bottle. Allow 10 to 14 days before harvesting the supernatant. Hybridomas are not efficient in immunoglobulin secretion during log phase growth. Allow the culture to enter stationary phase and, eventually, death phase. Maximum immunoglobulin concentration is reached after 10-14 days when no more live cells remain. Spin the culture at 670Xg for 10 minutes. Retain the supernatant and filter sterilize. Discard the cells. For 200ml of antibody supernatant, add 6ml of 1M Tris-HCL pH 7.5 buffer to stabilize the pH. A volume of antibody can be stored at 4°C to be used within two weeks and the remaining product diluted with an equal volume of reagent grade glycerol and stored at -20°C.

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