Cultivation of hybridoma cells

1. Thaw frozen at -70°C or lower temperature suspension cells. Place a vial immediately to water bath at 37°C for one minute until small piece of ice is left inside.

2. Before plating wash out DMSO. Transfer thawed cells in 10 ml of cell culture medium in 15-ml blue cap sterile tube, spin for 5 min at +4°C at 1000 rpm, discard supernatant and gently resuspend the pellet in 10 ml of fresh DMEM/F12 medium supplemented with 10-15% FBS.

3. Plate the cell suspension to 100 mm cell culture dish (BD, Falcon 100 x 15 mm style, cat # 351029). Optimal plating cell density is 1-3 x 10^6, the viability - 90% or higher. To check the cell viability mix 50 µl of cell suspension with 50 µl of 0.2% trypan blue in PBS and calculate number of stained (died) cell to the total cell number in a hemacytometer.

4. After two-three days of culture count the cells number and split the cells into 3-4 fresh culture dishes. Hybridoma cells can be removed from the plate by gentle pipetting and do not need to be trypsinized.

5. Repeat the splitting procedure several times until number of plates will reach the desired levels. 50-100 dishes are required to get finally 2 mg of the purified antibody (concentration of mAb in hybridoma culture supernatant is 10-50 µg/ml).

6. To freeze the hybridoma cells collect exponentially growing cells at >90% viability into 50-ml disposable sterile conical tube, spin the cells for 5 min at +4°C at 1000 rpm. Discard supernatant, resuspend cells in freshly made, sterile freezing medium (FBS with 10% DMSO), aliquot 1-2 ml samples in cryogenic vials, place the vials into foam box and keep them at -20C freezer for 1 hour, and then place into –70°C freezer. In a few day transfer vials to liquid nitrogen for time indefinite.

7. To grow hybridoma cells for antibody production cultivate cells until the medium is yellow and most of the cells are died. Collect suspension in 250-500 ml centrifuge containers, spin down at +4°C for 30 min with maximum speed, transfer the supernatants to fresh tubes and repeat spinning one more time. Use the supernatants to perform protein A, G, or L-affinity chromatography. For better results in affinity purification grow cells in serum free medium.