

## 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<sup>6</sup>, 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.