Protocol for Screening Mammalian Cells by Panning

Panning can be used to screen transfected mammalian cells using antibodies specific to membrane bound proteins. The procedure can be used to screen for positive cells (expressing the specific protein) or negative cells (which do not express the protein). If panning for a positive clone, it is important to use sterile reagents throughout the procedure.

Materials
- 100mm petri dishes
- PBS or HEPES buffer
- Fetal calf serum

1. Add 10ml of antibody solution to 100mm petri dish
2. Incubate at 45 minute at room temperature or overnight at 4°C.
3. Aspirate dish, wash twice with 5ml PBS or HEPES without serum.
4. Wash one time with 5ml of PBS or HEPES with 2% fetal calf serum.
5. Add 3×10^7 cells in 3ml of PBS or HEPES with 2% fetal calf serum.
6. Incubate for 90 minutes at 4°C with occasional swirling.
7. Remove non-adherent cells with a sterile pipet.
8. Carefully wash plate with three times with 5ml of PBS or HEPES with 2% fetal calf serum. Save and pool washes. To wash, slowly add the media down the side of the dish, swirl gently and slowly remove the media.
9. If doing a positive screen, add the appropriate growth media for cell growth. Grow cells at appropriate temperature.
10. If doing a negative screen, centrifuge cells from washes in Step 9 at 1,200 rpm for 5 minutes. Remove supernatant, add the appropriate growth media, resuspend the cells and plate. Grow cells at appropriate temperature.

Reagents & Solutions

Antibody Solution
- 20µg/ml of appropriate antibody in Panning buffer

Panning Buffer
- 50mM Tris-HCl, pH 9.5
- 0.15M NaCl

Reference

Eur. J. Immunology (1984) **14**:979 - 987.