

Fusion of B-lymphocytes and Myeloma Cells

B-lymphocyte - produces antibodies, but cannot survive in tissue culture beyond 7-10 days

myeloma cell - doesn't produce antibodies, but can survive in tissue culture "indefinitely"

fusion combines these two characteristics into one cell (a "hybrid")

B-lymphocyte (from spleen) + myeloma cell (from cultured cell line)



add 50% polyethylene glycol (PEG)
(fuses cell membranes)



two separate nuclei in one cell membrane



two nuclei will join (temporary tetraploid)



chromosomes will mix together



subsequent cell division (cells "return" to diploid nucleus)

some "hybrids" will have the desired mix of chromosomes

antibody specificity/antibody production + "immortality" in tissue culture

optimum fusion procedure - favors production of desired hybrids

cell ratio of 2 lymphocytes/1 myeloma cell (steps 1-4)

example: 4×10^7 lymphocytes mixed with 2×10^7 myeloma cells (2/1 ratio)

these number of cells will eventually fill all of the wells in one 96-well plate

50% **PEG concentration** (concentrations from 25%-65% have also been used by others)

exposure to PEG (steps 5-14) - *very* critical for good fusion

(lymphocytes+myeloma cells) + 50% PEG added very slowly with very gentle mixing

1 minute incubation (PEG is now in approximately 25% concentration)

PEG diluted out slowly (PEG is diluted to approximately 12% concentration) with very gentle mixing

1 minute incubation

PEG diluted slowly out to nearly 0% with very gentle mixing

optimum **cell density** in tissue culture after fusion (steps 17-18)

example: if you use 4×10^7 lymphocytes + 2×10^7 myeloma cells (in starting mixture before fusion)

then use 20ml HAT medium (function of HAT medium will be discussed next week)

these cell counts/ratio and amount of medium will fill all of the wells in one 96-well plate (200microliters/well requires about 20ml total to fill one plate)

you can use MORE lymphocytes and myeloma cells IF you have more cells than suggested above (but still maintain the 2:1 ratio)

you will need to adjust amount of HAT used if number of spleen and myeloma cells you use is *different* from the example given above (see lab procedure for volumes of HAT medium to use)

if you use more than 20ml of HAT medium, you will need more than one 96-well plate

maintain cells in tissue culture to:

allow non-fused B-lymphocytes to die (can NOT survive beyond 7-10 days maximum)

HAT medium will kill off any unfused myeloma cells

leaves alive ONLY hybrid cells (and a few fibroblasts, depending on how much connective tissue from spleen was ground up during the spleen cell preparation)

change medium every 4-7 days (steps 20-22) until vigorous growth of hybrids results

prevents nutrient depletion

maintains pH (along with CO₂)

discards waste products