# B Cell Fusion

## Preparation:

1. Pour 20 mls MEM (no FBS, no TC) into a 50 ml tube. Gas tube with 10% $CO_2$ and place in 37$^{o}$C water bath or bead bath.
2. PEG-8000 (40% polyethylene glycol 8000:60% MEM) is sterile filtered and in 1 ml aliquots. Place in 37$^{o}$C water bath.
3. Fill a 500 ml beaker with water and place in 37$^{o}$C incubator.

## Preparing spleen cells:

1. Tease out spleen cells in BSS.
2. Spin 5 minutes, 1500 rpm.
3. Wash 1X in BSS and count before final spin. Wash a 2$^{nd}$ time with the tumor cells.

## Preparing P3 or SP2/0 Myeloma Cells:

1. Spin 2 x $10^7$ SP2/0 cells for one fusion with $10^8$ spleen cells. If you have less spleen cells scale down the number of SP2/0 cells keeping the 5:1 ratio (spleen cell:SP2/0). BUT.... never use less than $10^7$ SP2/0 cells even if you have less spleen cells.
2. Wash 1X in BSS.
3. Combine with spleen cells for a second wash in BSS.
4. Aspirate off all supernatant. Do a 2 minute spin to get down all of the supernatant in the tube. Aspirate again.

## Fusion:

1. Break up cell pellet by gently tapping tube on table top.
2. Place tube in a beaker of fresh 37$^{o}$C water.
3. Add 1 ml PEG (37$^{o}$C) drop wise over 1 minute. Hold the tube at an angle and gently swirl to mix PEG and cells.
4. Allow tube to sit undisturbed for 1 minute at 37$^{o}$C.
5. Begin diluting out PEG with warm MEM: Add 1 ml MEM dropwise over 1 minute, twirling tube to mix. Make sure the PEG is being diluted and is not a clear layer on the cells.
6. Add 2 mls MEM over 1 minute. Mix.
7. Add 3 mls MEM over 1 minute. Mix.
8. Add 4 mls MEM over 1 minute. Mix.
9. Add 5 mls MEM over 1 minute. Mix.
10. Add another 5 mls MEM over 1 minute. Mix.
11. Place tube in 37$^{o}$C water bath for 10 minutes.
12. Spin 5 minutes, 1500 rpm.
13. Aspirate off supernatant from cell pellet.
14. Gently pour 50 mls BSS into tube. Do not resuspend the pellet!
15. Spin 5 minutes, 1500 rpm.
16. Aspirate off supernatant from cell pellet.

17. Prepare a bottle of 120 mls CTM (Complete Turmor Media) containing: recombinant IL-6 (500 Units/ml).
18. Using a 10 ml pipette slowly resuspend the fusion pellet in this CTM. Don't worry if there are a few clumps left in the pellet.
19. Plate out in 12 microtiter plates, 100 ul/well.
20. Place in 37$^{o}$C incubator, 10% $CO_2$.

## Feeding the fusion wells:

1. On day 1 add 50 ul/well of 3X HAT in CTM. This will give a final concentration of 1X HAT in each well. The stock of HAT is 50X. Dilute to 3X in CTM.
2. On day 5, 10 and 15: Aspirate off media (or dump the plate in sink) and add 100 ul/well of CTM + 1X HAT + rIL-6 (500 Units/ml).
3. Testing of B hybridomas can usually begin on day 10. If you are testing on the same day as feeding remember to test supernatants first, feed wells second.
4. After day 12 it is safe to transfer hybrids to CTM + 1X HT, and from there the next passage can be into CTM. **Never pass from CTM+ HAT directly into CTM alone. The cells must go through one passage in HT.**

## Making PEG 8000:

1. Weigh out 12 gm PEG 8000 powder + 18 mls MEM (40:60).
2. Tumble at room temp ~ 20-30 minutes.
3. Filter through 0.2 u sterile filter.

## HAT

10 mM sodium hypoxanthine
40 uM aminopterin
1.6 mM thymidine
Sigma H-0262. Dilute in 10 mls sterile CTM. Do NOT filter. Store at 4$^{o}$C.

## HT

100X HT supplement
10 mM sodium hypoxanthine
1.6 mM thymidine
Gibco #11067-030