T cell Fusion

Preparation:

1. Fill a 500 ml beaker with tap water and place in the 37^{o}C incubator.
2. Pour 50 mls MEM (no FBS) into a tube. Place in 37^{o}C water bath or bead bath.
3. Weigh out 10 gm PEG-1450.
4. Add 10 mls MEM to PEG. Tumble at room temp, until completely dissolved. Filter though 0.2 u filter. Place in 37^{o}C bead bath.
5. Spin 2 x 10^{7} BW/α-β- cells and 10^{8} T cell blasts. Combine cells into one tube and wash 2X in balanced salt solution (BSS). If you have less than 10^{8} T cell blasts you can reduce the number of BW's used (keep the 5:1 ratio the same). ***But never use less than 10^{7} BW's even if you have very few blasts.***

6. Aspirate off supernatant. Do NOT resuspend cells. Spin again, 1500 rpm, 3 minutes. This spins down any liquid on sides of tube. Remove all liquid by suction.

Fusion:

1. Break up cell pellet by gently tapping tube on desk.
2. Place tube in a beaker of 37^{o}C water.
3. Using a 1 ml pipette add 1 ml of the PEG/MEM mixture to the pellet over 15 seconds. Resuspend pellet with 1 gentle aspiration using the pipette. Allow tube to sit in the 37^{o}C water. This entire process should take 90 seconds.
4. Add 1 ml MEM (at 37^{o}C) down the side of the tube over 15 seconds very gently mixing as you do this.
5. Add 2 mls MEM for the next 15 seconds; then 3 mls MEM; then 4 mls MEM gently mixing after each addition.
6. Pour the remaining MEM down the side of the tube making sure the whole thing is very gently mixed.
7. Incubate the tube at 37^{o}C for 5 minutes.
8. Spin tube once 1500 rpm, 5 minutes.
9. Aspirate off supernatant. Gently pour 50 mls BSS down the side of the tube trying not to disrupt the pellet.
10. Spin tube 1500 rpm, 5 minutes.
11. Aspirate off supernatant.
12. Resuspend cells in 50 mls fresh CTM (Complete Tumor Medium) using a 10 ml pipette to break up the pellet. Plate out 40 mls in four 96-well microtiter plates, 100 ul per well (you can use a stepper with a 14g needle or a 12 channel pipetteman).
13. Add 40 mls CTM to the remaining 10 mls of cells. Mix gently, plate out four microtiter plates.
14. Repeat once more.
15. Place plates into 37^{o}C, 10% CO_2 incubator.

24 Hours Later:

1. Add 50 ul CTM + 3X HAT to each well. Final concentration of HAT= 1X.

Feeding Plates:

1. Four days later (5 days after fusion) feed wells with fresh CTM+HAT. This requires you to gently dump out the old medium from plates and add 100 ul fresh CTM+1X HAT.
2. By day 7 or 8 you should start to see wells with grown up hybrids. You can tell which wells are ready by using the naked eye. You can re-confirm the positive wells by then using the microscope. Transfer cells into a 1 ml Costar well with CTM+1X HAT. When the cells are half confluent in the wells they can be tested for function in a HT-2 assay or stained.
3. If cells test positively in a functional assay they can be transferred into a T-25 flask containing 10 mls CTM + 1X HAT. When they grow up freeze 2 vials from each T-25 flask.
4. After about day 12 it is safe to transfer hybrids to CTM + 1X HT, and from there the next passage can be into CTM. **Never passage cells from media with HAT into media without HAT or HT.**

HAT

10 mM sodium hypoxanthine
40 uM aminopterin
1.6 mM thymidine
Sigma H0262-10vl
This comes as a powder in a sealed vial. Add 10 mls sterile CTM to the vial and transfer into a sterile tube. Do NOT filter this solution. This makes a 50X HAT solution.

HT

100X HT supplement
10 mM sodium hypoxanthine
1.6 mM thymidine
Gibco #11067-030
This is a 100X HT solution in a small bottle.

PEG-1450 Sigma P-5402
Weigh out in tubes.
Store at room temperature.