Title: *Hybridoma Generation**: Cell Fusion** of myeloma SP2/0*

No: RTLP-GL-Ab-18

Location:	Approval Date:	Supersedes Date:
Old CCRC Tripp Lab	10 September 2004	

Materials:

- Lab coat
- Gloves
- RTLP # for Media
- RTLP # for Fusion
- RTLP # for ELISA
- **Prepared** SP2/0 cells*****
- 37°C H_2O bath
- Spleens from boosted animals
- Centrifuge
- Light microscope
- Hemacytometer
- Trypan blue
- 50 mL tubes
- Tissue Culture flasks
- 37°C Incubator
- 96-well microtiter plate
- Syringe plunger
- Mesh filter
- Pipettes
- Pipetteman
- Pipette Aid
- Pipetteman tips

***1 week prior!**

Procedure:

I. Cell Fusion and Hybridoma Selection:

1. **One week prior to fusion** prepare myeloma SP2/0 cells in DMEM-10%/HEPES/pyruvate, e.g. day 14 prior to final boost. At day of fusion, you will need ~10^8 SP2/0 cells/spleen to be fused
2. One day prior to fusion prepare fusion reagents:
   a) DMEM-10% containing 10mM HEPES +1 mM sodium pyruvate (cDMEM-10)
   b) DMEM-20% containing 10mM HEPES + 1 mM sodium pyruvate (cDMEM-20)
   c) Serum-free DMEM (SF-DMEM)
   d) SF-DMEM containing 10mM HEPES, and 1 mM sodium pyruvate (SF-cDMEM)
   e) 50% PEG, sterile
   f) ammonium chloride solution
   g) cDMEM-20 + 1x HAT
   h) cDMEM-20 + 1x HT

3. One day prior to fusion, split SP2/0 cells for vigorous growth

4. Day of fusion (per spleen):

a) Check SP2/0 cells for contamination and growth.

b) Pre-warm SF-DMEM, SF-cDMEM, cDMEM-20, and 50% PEG in a 37°C water bath.

c) Harvest spleens in SF-DMEM.

d) Dissociate spleens using mesh filter and wash in SF-DMEM.

e) Lyse RBCs using ammonium chloride solution, e.g. resuspend spleen cell pellet in 5 ml of ammonium chloride solution, incubate 5 min at RT, wash 2 times in SF-DMEM

f) Resuspend cell pellet in 10 ml of SF-cDMEM.

g) Wash 10^8 SP2/0 in SF-DMEM 2 times.

h) Resuspend cell pellet in 10 ml of SF-cDMEM.

i) Using trypan blue, count viable spleen cells.

j) Resuspend cells to 2.5 x 10^6 cell/ml in cDMEM-20.

6. Cell Fusion:

a) Mix SP2/0 cells and diluted spleen cells 1:1 in a 50 ml tube.

b) Centrifuge to wash and pellet cells.

c) Decant supernatant, and add 1 ml of pre-warmed PEG-50%, drop-by-drop over 1 minute, gently mixing the cells.

d) Stir gently for an additional minute.

e) Add 1 ml of pre-warmed SF-cDMEM to the cells, drop-by-drop over 1 minute, gently mixing the cells

f) Repeat once with an additional 1 ml of pre-warmed SF-cDMEM.

g) Add 7 ml of pre-warmed SF-cDMEM to tube, drop-by-drop with gentle stirring.

h) At this point you should see cell clumping.

i) Centrifuge lightly, 5 min at RT

j) Decant the supernatant, and forcefully discharge 10 ml of pre-warmed cDMEM-20 into pellet to break up clumps

k) Repeat "step j" several times to reach 40 mls of media, or roughly 2.5 x 10^6 cell/ml calculated from "step 4.j" above.

l) Place cells in a flask and incubate overnight at 37^{o}C to allow fibroblast to adhere and be removed from the hybridomas.

m) The next day, gently tap flask to dislodge hybridomas, and using a 10 ml pipet, add 2 drops/well of a 96-well plate using up all the volume of cells, and incubate overnight at 37^{o}C.

n) The next day, check seeded cells in wells. Wells should be nearly confluent.

o) Add 2 drops of cDMEM-20 + HAT/well using a 10 ml pipet and incubate at 37^{o}C .

p) BE EXTREMELY CAREFUL to avoid contamination, using separate pipets for each 96-well plate, and don't mix up plate lids.

q) On days 2-5, 7 and 9, aspirate half the volume of the plate well volume away, and replace with 2 drops/well cDMEM-20 + HAT from a 10 ml pipet – BE VERY ASEPTIC!

r) Profound cell death should occur between day 2 and 3 when HAT is added. Between days 7 and 9, viable hybridomas should be detected growing. The feeding schedule is rigid for days 2-5 post-fusion!

s) On day 14, repeat feeding except use cDMEM-20 + 1x HT.

t) On day 15, repeat feeding using cDMEM-20 only, and screen the little buggers for antibody by ELISA by collecting supernatant.

Author	Management Approval/Date	Effective Date