Standard Fusion Protocol

1. Grow myeloma cells few days prior fusion date. Myeloma cells culture should be maintained at <1x 10^6/ml density. Split myeloma cells the day before fusion to have next day 1x 10^7 cells (mouse splenocytes are fused with murine myeloma cell line at ratio 5:1);

2. Aseptically remove mouse spleen, place into sterile 100-mm cell culture plate containing 10 ml of sterile serum free DMEM/F12. Holding the spleen by sterile forceps perform spleen perfusion with serum free DMEM/F12 through 21g syringe needle. Pass splenocytes suspension through sterile Falcon 0.70 µm cell filter and resuspend cells into 15 ml tube. Centrifuge for 5 min and wash splenocytes two times in serum free DMEM/F12. Count cells;

3. Concurrently with the splenocytes wash myeloma cells twice with serum free pre-warmed DMEM/F12 and centrifuge for 5 min at 800 rpm remove all traces of fetal calf serum (FCS); Count cells;

4. Add an appropriate number of myeloma cells to half volume of spleen cells and centrifuge together for 5 min at 1000rpm. (Immediately freeze down the remaining splenocytes);

5. Remove all supernatant with Pasteur and suspend cells. Gently disrupt the pellet by tapping the bottom of the tube. Place the tube in a 37° C water bath and keep it there during fusion. Pre-warm 50% PEG1500 to 37°;

6. Add 1ml of warmed PEG 1500(w/v) to the pallet drop by drop over a period 1 minute while tapping the side of the tube to achieve thorough mixing. Continue to mix over next 1 minute at 37° C;

7. Dilute the PEG/cell mixture gradually as described below, continually but gently swirling the tube:

1ml serum free DMEM/F12 for 30-60 sec

4ml serum free DMEM/F12 for 3 – 4 min

20 ml serum free DMEM/F12 at once

20 ml DMEM/F12+20%FBS at once

8. Centrifuge fused cells for 5 min at 800rpm. Remove supernatant and gently resuspend the cells. Mix with the appropriate volume of DMEM/F12 supplemented with 20% FBS and HAT selection medium and plate 120 µl of cell suspension to six 96-well plates.

9. Fusion plates are examined visually at 24-48 after fusion for any abnormalities (i.e. contamination). At day 7 HAT selection medium have to be removed and cells are fed with fresh medium. After that cultures are examined every other day and fed. Once a majority of wells appear 50% confluent for growth the fusion products are screened by ELISA.