

Fusion Protocol

1. Prepare fusion partner (e.g. P3x63.Ag8 or NSO^{bcl-2}) to be growing in log phase for day of fusion.
2. About 1 hr before fusion, put 1 bottle of DMEM (500ml) AND 1 BOTTLE OF peg 1500 (Roche, #14982000, 4ml vial, 50% stock) in 37°C water bath.
3. Sterily prepare splenocytes from immune mouse or rat, wash x1 in warm DMEM, count cells.
4. Obtain accurate counts of splenocytes and myeloma fusion partner cells, each rinsed and suspended in DMEM. Usually for one spleen use 20×10^6 myeloma cells.
5. Determine the desired ratio of splenocytes:fusion partner you wish to use so as to obtain approximately 1 viable fusion product per well. This can vary between 1:1 or 5:1. We typically use 3:1. Also, when plating out fusion products into 96well plates, we plate out at $2-3 \times 10^5$ splenocytes per ml (using NSO^{bcl-2} partner) and 1×10^6 splenocytes per ml (using P3x63.Ag8 partner), plating 100ul/well.
6. Mix appropriate number of splenocytes and partner cells in 50 ml tube and spin down at 1200rpm for 10 min.
7. Remove supernatant and loosen cell pellet by gently tapping the bottom of the tube.
8. Slowly (over the course of 90 seconds) add drop-wise 2 ml of **warmed** PEG 1500 solution, while gently agitating cells.
9. Wait for 30 seconds.
10. Slowly (over the course of 5 min.) add drop-wise 40 ml of **warmed** DMEM, while gently agitating cells.
11. Spin at 1200 rpm for 10 min.
12. Resuspend fused cells in HAT media in appropriate volume and plate 100ul/well
13. Aprox. 3 days after plating, feed cells with additional 50ul HAT media per well
14. Hybridoma should be ready to screen 10-14 days post fusion. Ideally the should be only a single cluster of cells (representing a “clonal” hybridoma) per well. This will aid in identifying positive clones and minimize the risk of a positive clone being out-competed by a negative hybridoma clone residing in the same well.
15. Once a positive clone has been identified, it must be sub-cloned by Limiting Dilution using Cloning media (the NSO^{bcl-2} cells are fed with HAT for at least 3 weeks after fusion).