LIF Conditioned Media Protocol

The LIF was produced from HEK293 cells in 10%FBS MEM**.** I made a stable producer cell line. The LIF gene was cloned by Rt-PCR of human RNA and modified by tagging of V5 at c-terminal. Otherwise, it was the same as the human one. The LIF producer HEK293 cells were stable transfected with pcDNA3.1(neo) harboring the LIF gene tagged with V5. (The recombinant LIF can be easily detected from the medium by V5 western. It can be selected with G418 and the expression level can be measured by Western blotting of V5 tag. Allow to grow for 3 days before collecting Cm, by which time point cells are fully confluent. Filter CM with 0.22μm filter.

Treat the 9/3AH cells with LIF CM, produced from HEK293 cells and mixed with fresh medium at 1:1, 1:2, and 1:4 ratio (CM: fresh medium), in parallel with the control CM mixed at the same ratio. You can also use the recombinant LIF (Chemicon, Cat# LIF1010, Lot#1111108) at 40, 80, 160 ng/ml.

I observed the cells morphological changes up to 6 days. 9/3AH cells did not respond to LIF CM or recLIF as quickly as MPC862L cells did, but by day 3, I could see 9/3AH cells changing morphology responding specifically to LIF; I could not see any change induced by the control CM. By day 6, the changes became very clear. LIF CM of recLIF treated 9/3AH ccells showed more flattened shape and increased neuritis processing similar to 862L cells. I saw most significant effects with CM mixed at 1:4 (LIF CM: fresh medium) ratio and with recLIF at 40 mg/ml. LIF CM and recLIF did not show any difference.