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Metabolic Labeling and Immunoprecipitation of VEGF Isoforms

Adapted from (Park et al., *The Vascular Endothelial Growth Factor (VEGF) Isoforms: Differential Deposition into the Sub epithelial Extracellular Matrix and Bioactivity of Extracellular Matrix-bound VEGF*. Molecular Biology of the Cell, 4:1317-1326, 1993.)

1. Plate T47D cells in duplicate at different densities: 2.5×10^5 , 5×10^5 , 7.5×10^5 cells per well in a 6 well plate. Incubate at 37°C overnight.

Next day:

2. The next day, aspirate media from the well. Replace with DMEM (-Met, -FBS) to wells. Allow cells to incubate for 5 hours.

3. Observe the cells. Should use cells that are ~60% confluent. I found that 5×10^5 cells/well was good for all cell types, except T47D-PCDNA, where I used 7.5×10^5 cells/well.)

4. Aspirate Media. Add $1000 \mu\text{Ci}$ to 9 mL DMEM media (-Met, -FBS). Each well received 1 mL hot media, or $111 \mu\text{Ci}$ total per well. Concentration should be $\sim 10 \mu\text{Ci}/\mu\text{L}$.

5. Incubate overnight (-18.5 hours).

Next day:

6. Remove media and aliquot into tubes for further analysis. Remove cell monolayer with EDTA, store in tubes. Use 1 mL RIPA buffer (1% deoxycholate, 0.1% sodium dodecylsulfate (SDS), 0.9% NaCl, 25 mM tris(hydroxymethyl) aminomethane (Trish), 1 mM EDTA pH 7.4) to extract ECM layer. Centrifuge media and ECM samples to remove debris.

7. Add $20 \mu\text{L}$ of anti-human VEGF antiserum and mix for 1 hour.

8. Add $50 \mu\text{L}$ Protein A agarose and incubate for -4 hours.

9. Pellet beads and wash 4 times with RIPA buffer.

10. Add $50 \mu\text{L}$ sample buffer to each sample, boil.

11. Run at 120 mV in 20% Tricine gel for 4.5 hours.

12. Fix in 10% Acetic Acid, 30% MeOH for 30 min.
13. Soak in excess dH₂O for 30 min.
14. Agitate in Fluoro-Hance for 30 min. Gel should be floating.
15. Remove gel, place on Saran Wrap. Cover with filter paper. **Dry** for 2 hours in the gel dryer (at 65°C).
16. Expose at 80 °C for 2-5 days with Kodak MS film.