PROTOCOL

Conditioned Media Sample Preparation

1. Grow cells of interest at relatively high density (110, 000 cells/cm^2). I usually use 5 x 10-cm dishes to yield ~50 ml of conditioned media.
2. Add culture media to an identical number of culture dishes treated identically but do not add any cells to these dishes. This media will serve as the negative control.
3. Change the media to serum-free growth media and allow cells to condition the media for 24-48 hours. Change media on the control plates as well.
4. Collect the media from all of the plates, centrifuge at 1000g for 10 min and carefully remove the supernatant, leaving a small quantity of media behind. The point of this step is to make sure there are no cells in the media. Combine all of the media from each of the 5 plates to yield 50 ml of conditioned media and 50 ml of control media.
5. Pre-wash an Amicon Ultra-15 centrifugal filter (3 kDa MWCO) with 50 mM ammonium bicarbonate pH 8 for 10 min at 3500 rpm.
6. Add 15 ml of the conditioned media to the Amcion and spin at 3500 rpm for 30 minutes. Repeat these intervals until the volume is reduced to 1-2 ml.
7. Fill Amicon with 50 mM ammonium bicarbonate pH 8.
8. Spin Amicon at 3500 rpm for 30 min intervals until the volume reduces to ~1-2 ml.
9. Repeat steps 7 and 8 until the final volume is ~250 ul and there is no pink colour (from the phenol red) that is detectable by eye. Usually takes at least 5 repeats.
10. Determine protein concentration
11. Lyophilize solution until dry