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Cell culture: Harvesting secreted proteins in conditioned medium

Need to reach 80% confluency (80% of the surface of a culture vessel is covered with cells) before changing media and harvesting secreted proteins. To remove additives that may interfere with downstream proteomics analysis (ex. FBS) it is of vital importance that the cell medium is purchased without these additives.

Culture flask T75 (75 cm2 growth area), expected yield: 10-20 µg secreted proteins
Culture flask T175 (175 cm2 growth area), expected yield: 20-60 µg secreted proteins

Cell washing

1. Wash cells with PBS 3 times and cover with 15 mL medium without additives (NO SERUM) for 1 hour.
2. Wash cells again with PBS 3 times.

Incubation, collection of secreted proteins

3. Add 15 ml phenol- and serum-free media complete with l-glutamine and antibiotics and incubate for 24-48 hours.
4. Filter the medium through a 0.2 µm syringe to remove cellular debris.
5. Transfer the conditioned media to 15 mL Nunc tubes and centrifuge at 3000 x g at 4 °C for 5 minutes to remove cell debris (supernatant may be stored at – 80 °C prior to further analysis).
6. Concentrate the conditioned medium 40-50 times through centrifugation at 4 °C, 4000 x g, using Amicon Ultra-15 3 kDa MWCO filters (Millipore).
7. Add 10 mL of MilliQ water to the filter and repeat centrifugation.
8. Add 10 mL of MilliQ water to the filter and repeat centrifugation.
9. Transfer the concentrate on top of the filter to 1.5 mL tubes (Protein LoBind, Eppendorf), and measure the protein concentration using Qubit. Lyophilize prior to sample preparation for MS analysis

1L PBS (Phosphate Buffered Saline) pH 7.4

Dissolve 8g NaCl, 200mg KCl, 1.44g Na2HPO4, and 245 mg KH2PO4 in 800 ml distilled water. Adjust pH to 7.4 and top up to 1L with water.

PBS can also be purchased from vendors like Sigma Aldrich (art.no. D8537) and VWR (art.no. K812)