Cell culture supernatant preparation:

1. Centrifuge to Remove Particles:

- Centrifuge samples at 1400 RPM for 10 minutes at 4°C to eliminate particles.

2. Aliquoting Clarified Medium:

- Aliquot the clarified medium into clean polypropylene microcentrifuge tubes.
- Use immediately or store aliquots at -80°C. Avoid multiple freeze/thaw cycles.

3. Normalization Process:

- Normalize by plating an equal number of cells.
- If the experiment exceeds 24 hours, count live cells at the end for normalization.

4. Sub-aliquoting and Media Submission:

- Sub-aliquot the supernatant into frozen portions for future assays.
- Submit an aliquot of the media for background level.

5. Sample Submission:

- When submitting samples, run singlets and provide independent biological replicates.
    - Use tubes/vials (low binding) or 96-well polypropylene plates (low protein binding).
    - Attach a clear and readable Excel list matching your plates or vials labels.