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# Quick protocol for protein extraction from adherent fish-derived fibroblasts

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**Protocol status:** Working
**We use this protocol and it's working**

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## Disclaimer



## Abstract

We present a rapid and efficient protocol for extracting total proteins from adherent fish-derived fibroblasts, specifically optimized for applications in Western blotting and mass spectrometry. The method involves directly dissociating cells from the culture flask into a lysis buffer (e.g. RIPA buffer) followed by centrifugation to collect the total soluble proteins. The use of buffers like RIPA ensures comprehensive solubilization of cellular proteins while preserving their integrity and functionality. This straightforward and reproducible protocol yields high-quality protein extracts suitable for various downstream analytical techniques. Its simplicity and reliability make it an invaluable tool for researchers studying proteomics using fish cell culture.

## Materials

### Solutions

- 1X Phosphate Buffered Saline (PBS)
- RIPA lysis buffer: 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1mM EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, sterile-filtered.

### Materials

- 2 mL or 1.5 mL microcentrifuge tubes
- Cell scrappers
- Pipettes and pipette tips
- Serological pipettes (variable volume)
- Pasteur pipettes

### Equipments

- Refrigerated microcentrifuge
- Flow hood chamber

## Troubleshooting

1 Carefully remove all culture media from the flask and add enough ice-cold 1X PBS to wash the cells

2 Carefully remove the ice-cold 1x PBS and add ice-cold lysis buffer (RIPA buffer) according to the estimated number of cells:
1 mL for $10^7$ cells (roughly a T75 flask).

3 Use a cell scraper to dissociate the cells from the bottom of the flask.

4 Resuspend the cells in the lysis buffer and transfer the suspension to a microcentrifuge tube.

5 Agitate for 20 minutes at 4ºC.

6 Centrifuge at 13,000 x g for 20 min at 4°C.

7 Carefully transfer the supernatant containing the soluble protein to a new tube and keep on the ice. Discard the pellet.

Note: The protein solution can be kept in the freezer for longer storage periods until further use.