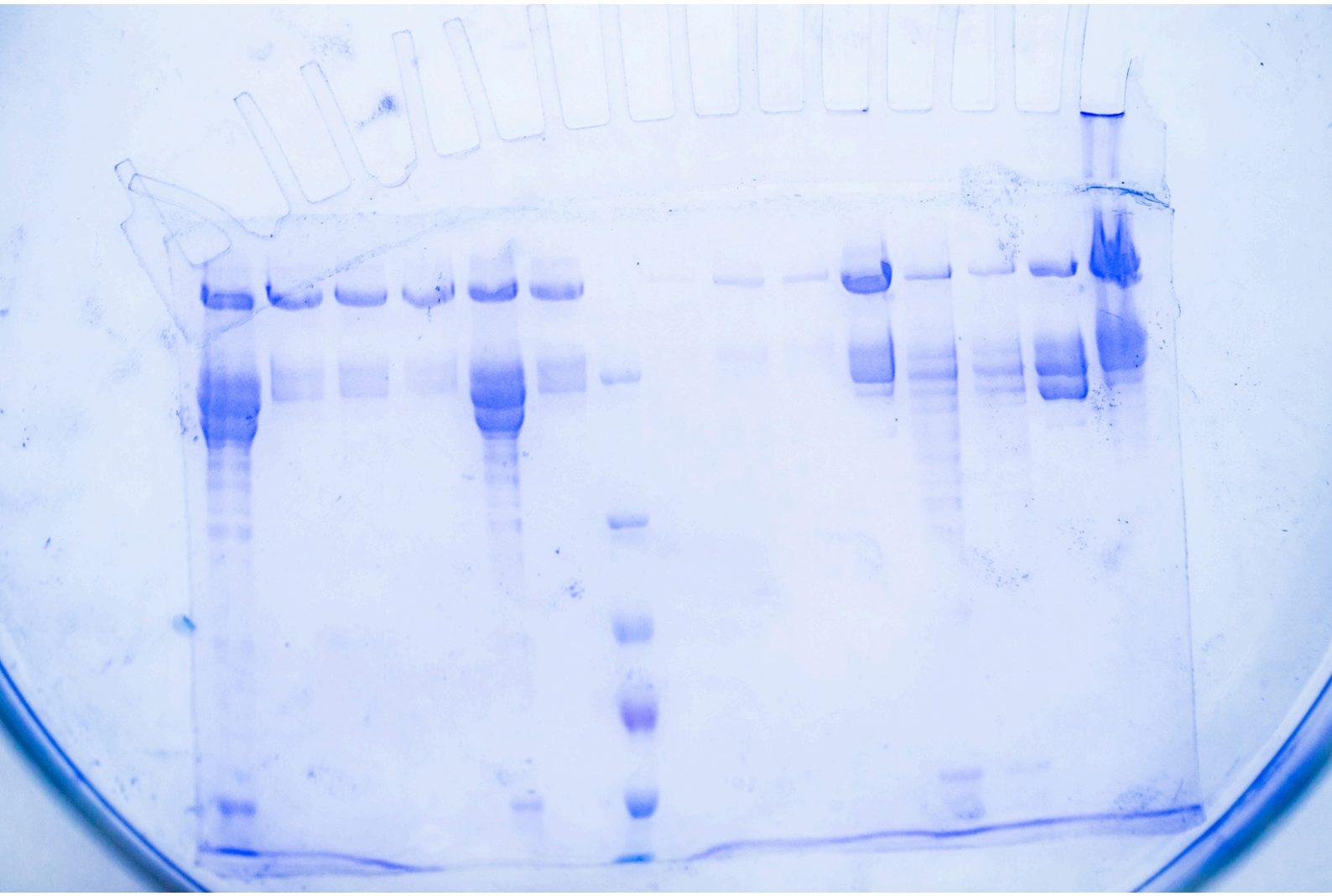
Immunoprecipitation



Solutions and Reagents

Preparation of Cell Lysates

Pre-clearing

Immunoprecipitation (IP)

Protocol Summary

Solutions and Reagents

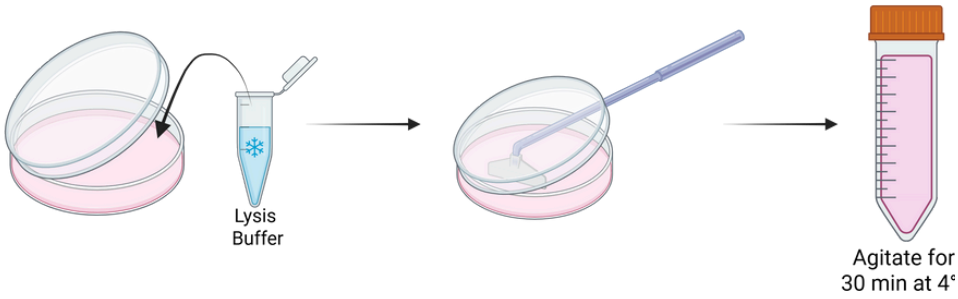
Lysis buffer:

- Use RIPA buffer (25 mM Tris-HCl pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS). Proteinase inhibitor cocktail should be added fresh before each use.
- The usage of SDS depends on the nature of the cells. For some cell lines, 0.1% SDS will release DNA and thus make it hard to extract proteins out. In those cases, omit SDS.

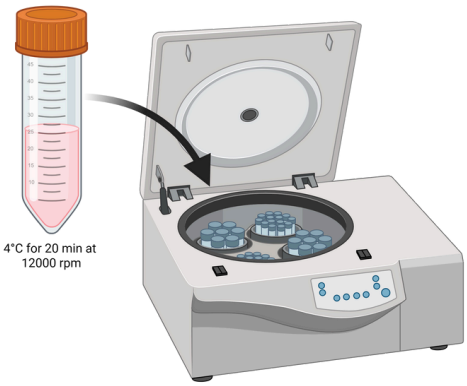
Preparation of Cell Lysates

Steps

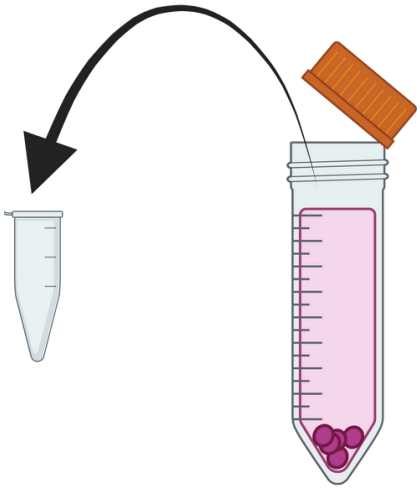
1. Add ice cold lysis buffer (1ml per 100mm-dish or 10^7 cells, or adjust based on your specific requirements). Scrape off cells (for adherent cells still on plate) and resuspend cells. Collect cells in a centrifuge tube and agitate for 30 min at 4°C.



2. Spin cells at 4°C for 20 min at 12000 rpm.



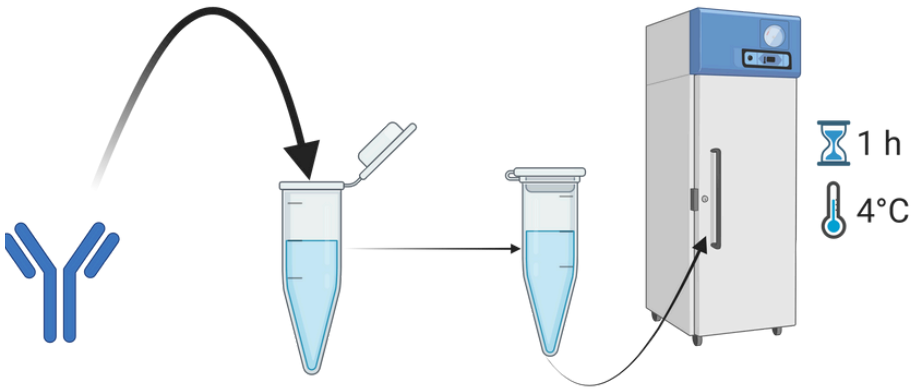
3. Save the supernatant which is the cell lysates.



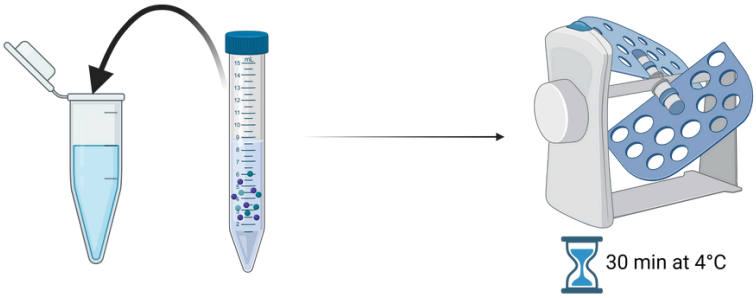
Pre-clearing

Steps

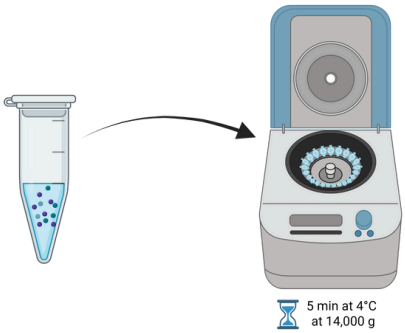
1 Add normal serum or an irrelevant antibody that matches the species and isotype of the antibody you plan to use for immunoprecipitation (IP). Use at least 5 times the amount of the IP antibody. Incubate the mixture for 1 hour at 4°C.



2 For 1 ml lysate, add 100 ul of protein A or protein G beads slurry (50 ul solid bed volume), and incubate at 4°C for 30 min on a rotator.



3 Spin down beads at 14000g for 5 min at 4°C.

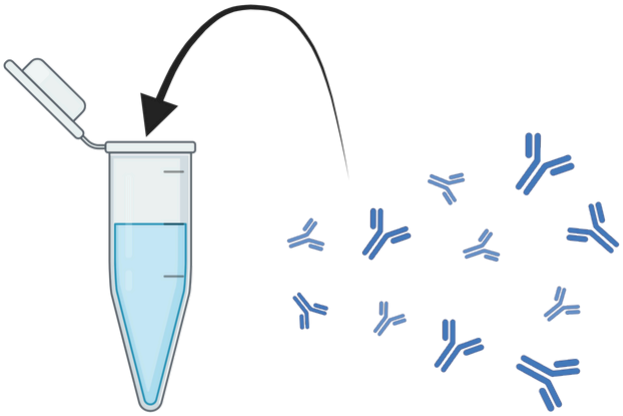


4 Save the supernatant which is the pre-cleared lysates.

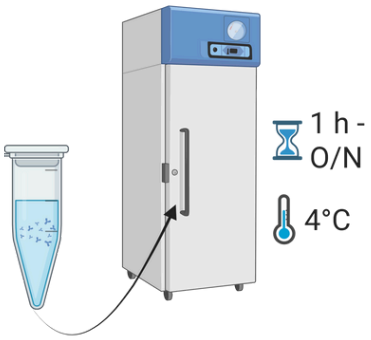
Immunoprecipitation (IP)

Steps

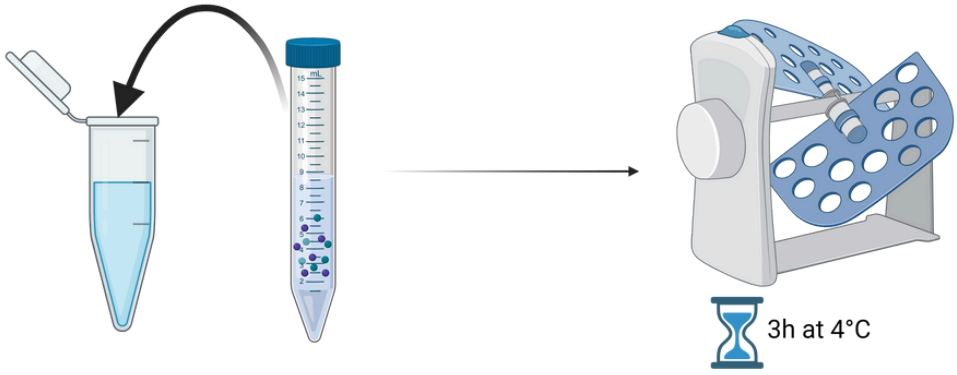
1 Add IP antibody to the pre-cleared lysates. You will need to determine the best amount of antibody to use. As a starting point, you may use 1 ug antibody for every ml of lysates.



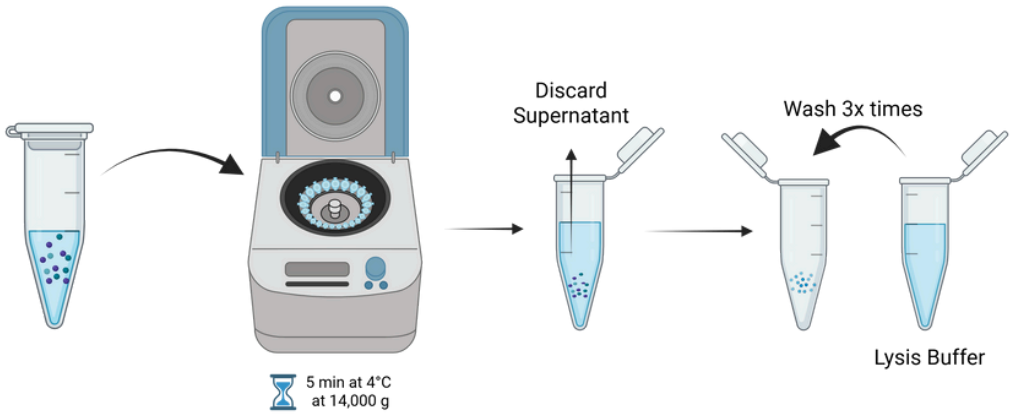
2 Incubate for a certain amount of time (from 1 hr to overnight, depending on your specific conditions) at 4°C.



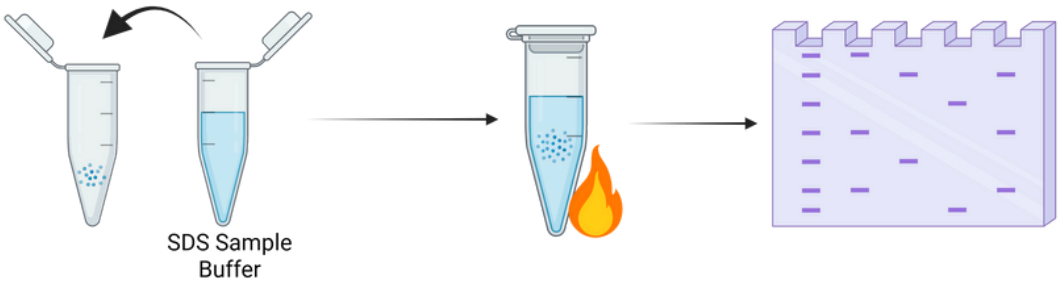
3 Add 100 ul of protein A or protein G slurry (50 ul solid bed volume) to 1 ml lysate and incubate for 3 hr at 4°C on a rotator.



4 Spin down beads, remove supernatant, and wash beads 3 times with lysis buffer.



5 Add SDS-PAGE sample buffer to beads. Boil and run gel.



Immunoprecipitation Protocol Overview

Preparation of Cell Lysates:

- Add ice cold lysis buffer (1ml per 100mm-dish or 10^7 cells, or adjust based on your specific requirements). Scrape off cells (for adherent cells still on plate) and resuspend cells. Collect cells in a centrifuge tube and agitate for 30 min at 4°C.
- Spin cells at 4°C for 20 min at 12000 rpm.
- Save the supernatant which is the cell lysates.

Pre-clearing:

- Add normal serum or irrelevant antibody from the same species and isotypes as the IP antibody you will use. The amount should be at least 5-fold more than the amount you will use for IP. Incubate for 1 hr at 4°C.
- For 1 ml lysate, add 100 ul of proteins A or protein G beads slurry (50 ul solid bed volume), and incubate at 4°C for 30 min on a rotator.
- Spin down beads at 14000g for 5 min at 4°C.
- Save the supernatant which is the pre-cleared lysates.

Immunoprecipitation (IP):

- Add IP antibody to the pre-cleared lysates. You will need to determine the best amount of antibody to use. As a starting point, you may use 1 ug antibody for every ml of lysates.
- Incubate for a certain amount of time (from 1 hr to overnight, depending on your specific conditions) at 4°C.
- Add 100 ul of protein A or protein G slurry (50 ul solid bed volume) to 1 ml lysate and incubate for 3 hr at 4°C on a rotator.
- Save the supernatant which is the pre-cleared lysates. Spin down beads, and remove supernatant.
- Wash beads 3 times with lysis buffer.
- Add SDS-PAGE sample buffer to beads. Boil and run gel.

Notes

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