

As with all aspects of assay development, optimum sample preparation procedures will vary depending on the target and assay of interest. It is always good practice to consult the literature for experimental examples similar to your own when developing a new assay.

**ELISA**

**Sample preparation  
methods**

# Cell culture supernatant

- Pipette cell culture media into a centrifuge tube and centrifuge at 1,500 rpm for 10 min at 4°C.
- Immediately aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

# Cell extract

- Place tissue culture plates on ice.
- Aspirate medium and gently wash cells once with ice-cold PBS.
- Aspirate PBS and add 0.5 mL complete extraction buffer per 100 mm plate.
- Scrape cells to collect in tilted plate and remove to pre-chilled tube.
- Vortex briefly and incubate on ice for 15-30 min.
- Centrifuge at 13,000 rpm for 10 min at 4°C to pellet insoluble contents.
- Aliquot supernatant (this is the soluble cell extract) to clean, chilled tubes on ice and store samples at -80°C. Minimize freeze/thaw cycles.

# Conditioned medium

- Place cells in complete (serum-containing) growth medium and allow cells to proliferate to desired level of confluence.
- Remove growth medium and wash very gently with a few mL of warm PBS. Repeat wash step.
- Remove last PBS wash and gently add serum free growth medium.
- Incubate 1-2 days.
- Pipette medium into a centrifuge tube and centrifuge at 1,500 rpm for 10 min at 4°C.
- Immediately aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

# Milk

- Collect samples and centrifuge at 10,000 x g for 2 min at 4°C.
- Aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

# Plasma

- Collect whole blood into anti-coagulant containing tube, such as BD Vacutainer Coagulation tubes or add 0.1 M sodium citrate to 1/10 final volume.
- Centrifuge at 3,000 rpm for 10 min at 4°C.
- Immediately aliquot supernatant (plasma) and store samples at -80°C. Minimize freeze/thaw cycles.

# Urine

- Collect samples and centrifuge at 10,000 x g for 2 min at 4°C.
- Aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.

# Saliva

- Collect samples and centrifuge at 10,000 x g for 2 min at 4°C.
- Aliquot supernatant and store samples at -80°C. Minimize freeze/thaw cycles.



# Serum

- Collect whole blood in untreated test tube or, for example, an anti-coagulant free tube such as BD Vacutainer Serum tubes.
- Incubate undisturbed at room temperature for 20 min.
- Centrifuge at 3,000 rpm for 10 min at 4°C.
- Immediately aliquot supernatant (serum) and store samples at -80°C. Minimize freeze/thaw cycles.



# Tissue extract

- ◉ Dissect the tissue of interest with clean tools, on ice preferably and as quickly as possible to prevent degradation by proteases.
- ◉ Place the tissue in round bottom microfuge tubes and immerse in liquid nitrogen to "snap freeze". Store samples at  $-80^{\circ}\text{C}$  for later use or keep on ice for immediate homogenization.
- ◉ For a  $\sim 5$  mg piece of tissue, add  $\sim 300$   $\mu\text{L}$  complete extraction buffer (see cell/tissue extraction buffer recipe) to the tube and homogenize with an electric homogenizer.
- ◉ Rinse the blade twice using  $300$   $\mu\text{L}$  complete extraction buffer for each rinse, then maintain constant agitation for 2 hr at  $4^{\circ}\text{C}$  (e.g. place on an orbital shaker in the cold room).
- ◉ Centrifuge for 20 min at 13,000 rpm at  $4^{\circ}\text{C}$ . Place on ice, aliquot supernatant (this is the soluble protein extract) to a fresh, chilled tube and store samples at  $-80^{\circ}\text{C}$ . Minimize freeze/thaw cycles.

# Cell/tissue extraction buffer recipe

- 100 mM Tris, pH 7.4
- 150 mM NaCl
- 1 mM EGTA
- 1 mM EDTA
- 1% Triton X-100
- 0.5% Sodium deoxycholate
- Additional reagents required to produce **complete** extraction buffer.
- Phosphatase inhibitor cocktail
- Protease inhibitor cocktail
- PMSF

# General recommendations

- Recommended protein extract concentration is at least 1-2 mg/mL.
- Typically, serum, plasma, cell and tissue extracts are diluted by 50% with binding buffer.
- Prior to use after thawing, centrifuge samples at 10,000 rpm for 5' at 4°C to remove any precipitate.

# References

- ① <https://www.abcam.com>
- ② <https://www.sinobiological.com>
- ③ <https://www.bio-rad>
- ④ <https://www.thermofisher.com>