Sample collection guides

A guide to ELISA sample collection

Introduction

These ELISA sample collection guidelines are intended preparing commonly tested samples for use in ELISA assays. Specific protocols may vary by cell line or tissue type. Avoid repeated freeze-thaw cycles for all sample types. Please refer to the protocol for product-specific details regarding sample preparation and compatible sample types.

Sample collection

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. After mix 10 ~20 minutes, centrifuge samples for 20 minutes at 2000 ~3000 rpm. Collect the supernatant without sediment.

Serum

The samples should be allowed to clot in the collection tubes for a minimum of 30 minutes at room temperature. Serum should be separated from the clot by centrifuging the collection tube for 20 minutes at 2000 ~3000 rpm.

Cell Culture Supernatants

Collect by sterile tubes. When detecting secrete components, centrifuge at 2000 ~3000 rpm for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 rpm for 20 minutes. Collect the supernatant without sediment.

Tissue Homogenates

Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 15 minutes at 12,000 rpm at 4 °C to get the supernatant. Avoid freeze/thaw cycles.

Saliva

Collect saliva in a tube and centrifuge for 5 minutes at 2000 ~3000 rpm for 20 minutes. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid freeze/thaw cycles.

Cell Lysates

Solubilize cell in lysis buffer and allow to sit on ice for 30 minutes. Centrifuge at 13,000 rpm for 10 minutes to remove insoluble material. Aliquot the supernatant into a new tube and discard the remaining whole cell extract. Quantify total protein concentration using a total protein assay. Assay immediately or aliquot and store at ≤ -20 °C. Avoid freeze/thaw cycles.

Urine

Collect fresh urine into a sterile or disposable container. Centrifuge sample at 1,000 ~2,000 rpm for 5 minute. Assay immediately or aliquot supernatant and hold at -80°C. Avoid freeze/thaw cycles.

Bronchoalveolar lavage fluid/Synovial fluid

Centrifuge samples for 15 minutes at 2000 ~3000 rpm to remove particulate. Collect the supernatant and freeze at -20°C.

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°C for later use or keep on ice for immediate homogenization.
- ✓ For a ~5 mg piece of tissue, add ~300 µL complete extraction buffer (see cell/tissue extraction buffer recipe) to the tube and homogenize with an electric homogenizer.
- ✓ Rinse the blade twice using 300 µL complete extraction buffer for each rinse, then maintain constant agitation for 2 hours at 4°C (e.g. place on an orbital shaker in the cold room).
- ✓ Centrifuge for 20 minutes at 13,000 rpm at 4°C. Place on ice, aliquot supernatant (this is the soluble protein extract) to a fresh, chilled tube and store samples at -80°C. Minimize freeze/thaw cycles.

Volumes of lysis buffer must be determined in relation to the amount of tissue present. Typical concentration of final protein extract is >1 mg/mL.

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

Supplement the cell extraction with phosphatase and protease inhibitor cocktails as described by manufacturer, and PMSF to 1 mM, immediately before use.

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 x rpm for 5 minutes at 4°C to remove any precipitate.

If your sample for ELISA assay is not listed above, please contact our technical team: support@bt-laboratory.com for more instructions.