Sample Preparation and Handling

Please select the sample type validated with your assay (see Chapter 1 of the datasheet).

1. Prepare serum

- Collect whole blood in a covered test tube.
- After collecting whole blood, allow the blood to clot by leaving it undisturbed at room temperature (usually 15 –30 minutes).
- Remove the clot by centrifuging at 1000 –2000 × g for 10 minutes in a refrigerated centrifuge.
- The resulting supernatant is serum. Immediately transfer the serum into a clean polypropylene tube using a Pasteur pipette.
- Maintain the samples at 2 to 8°C while handling. If the serum is not analyzed immediately, aliquot the serum into 0.5 mL aliquots. Store and transport the serum aliquots at –20°C or lower.

Note: Avoid freeze-thaw cycles because this is detrimental to many serum components.

2. Prepare plasma

- Collect whole blood into commercially available anticoagulant-treated tubes e.g., EDTA-treated or citrate-treated.

Note: Heparinized tubes are indicated for some applications; however, heparin may be contaminated with endotoxin, which can stimulate white blood cells to release cytokines.

- Remove cells from plasma by centrifugation for 10 minutes at 1000 –2000 × *g* using a refrigerated centrifuge.
- Centrifuge for 15 minutes at 2000 × *g* to deplete platelets in the plasma sample.
- The resulting supernatant is designated plasma. Immediately transfer the plasma into a clean polypropylene tube using a Pasteur pipette.
- Maintain the samples at 2 to 8°C while handling. If the plasma is not analyzed immediately, aliquot the plasma into 0.5 mL aliquots. Store and transport the plasma aliquots at –20°C or lower.

Note: Avoid freeze-thaw cycles.

3. Prepare tissue culture medium

- Following the end of the desired cell culture time, pipette medium into a microcentrifuge tube and immediately place the tube on ice.
- Centrifuge the sample at 1400 rpm for 1 minute.
- Transfer supernatant and aliquot into a clean microcentrifuge tube.
- Store at –80°C until ready for use in ELISA.

4. Nuclear extraction method

This protocol has been successfully applied to several cell lines of human origin.

- Prepare the recommended 1X Hypotonic Buffer:
  - 20 mM Tris-HCl, pH 7.4
  - 10 mM NaCl
  - 3 mM MgCl2
- Collect cells (5 × 106) in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
- Wash cells twice with cold PBS.
- Remove and discard the supernatant and transfer the cells into a prechilled microcentrifuge tube.
- Gently resuspend cells in 500 µL 1X Hypotonic Buffer by pipetting up and down several times. Incubate on ice for 15 minutes.
- Add 25 µL detergent (10% NP40) and vortex for 10 seconds at highest setting.
- Centrifuge the lysate for 10 minutes at 3000 rpm at 4°C.
- The supernatant contains the cytoplasmic fraction (save the supernatant). The pellet is the nuclear fraction.
- Resuspend nuclear pellet in 50 µL Cell Extraction Buffer with protease inhibitors (page 16) for 30 minutes on ice with vortexing at 10 minute intervals.
- Centrifuge for 30 minutes at 14,000 × g at 4°C. Transfer supernatant (nuclear fraction) to a clean microcentrifuge tube.
- Aliquot and store the nuclear extracts at –80°C until use.

5. Cell extraction protocol

- Prepare the required amount of Cell Extraction Buffer with the following protease inhibitors just prior to use:
  - 1 mM PMSF (stock is 0.3 M in DMSO)
  - Protease inhibitor cocktail (e.g. Sigma Cat. no. P-2714, reconstitute according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

Note: The stability of Cell Extraction Buffer with protease inhibitors is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

- Estimate cell density for suspension cells by counting in a hemacytometer. Estimate cell density for adherent cells by visual inspection under a microscope. Use cells at 70–80% confluence levels.
- Stimulate cells as desired.
- Transfer the cells into clean 15 mL conical tubes:
- Collect the cells by centrifugation at 300 × g for 7 minutes. Aspirate the medium.
- Resuspend the pellet in ice-cold PBS.
- Collect the cells by centrifugation at 300 × g for 7 minutes at 4°C. Aspirate the PBS.
- Lyse the cells by pipetting Cell Extraction Buffer with protease inhibitors from step 1 into each tube. Use 1 mL Complete Cell Extraction Buffer per 108 cells.
- Transfer the lysates to clean microcentrifuge tubes.
- Vortex the mixture, then incubate the mixture on ice for 30 minutes, with occasional vortexing.
- Clarify the lysates by centrifugation at 13,000 × g at 4°C for 10 minutes.
- Transfer the clarified cell extracts to clean microcentrifuge tubes.
- Store the clarified cell extracts at –80°C until use. Avoid repeated freezethaw cycles.

Determine protein concentration using a suitable method.

All cell extracts require dilution by a factor of at least 1:10 in Standard Diluent Buffer before analysis.

6. Tissue homogenate protocol

- Prepare the required amount of Cell Extraction Buffer with the following protease inhibitors just prior to use:

  - 1 mM PMSF (stock is 0.3 M in DMSO)
  - Protease inhibitor cocktail (e.g. Sigma Cat. no. P-2714, reconstitute according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

Note: The stability of Cell Extraction Buffer with protease inhibitors is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

- Add ~100 mg of the tissue sample (wet mass) to an Eppendorf tube.
- Add 8 volumes of Cell Extraction Buffer with protease inhibitors from step 1 to the tube by 50–100 µL increments, and homogenize thoroughly with a Dounce homogenizer or by sonication.
- Transfer the lysate to a clean microcentrifuge tube.
- Vortex the mixture, then incubate the mixture on ice for 30 minutes, with occasional vortexing.

- Clarify the lysate by centrifugation at 13,000 × g at 4°C for 10 minutes.
- Transfer the supernatant to a clean microcentrifuge tube.
- Determine protein concentration using a suitable method.
- Dilute the tissue extract an additional 1:10 to 1:100 with Standard Diluent Buffer before analysis.

7. Brain tissue homogenate protocol

- Prepare the required amount of homogenization buffer and protease inhibitors prior to use:
  - 5 M guanidine-HCl diluted in 50 mM Tris, pH 8.0
  - 1X PBS supplemented with 1X protease inhibitor cocktail.

- Add ~100 mg of the brain tissue sample (wet mass) to an Eppendorf tube.
- Add 8 volumes of cold 5 M guanidine-HCl in 50 mM Tris to the tube by 50–100 µL increments, and grind thoroughly with a hand-held tissue homogenizer after each addition.
- (Optional) Transfer the homogenate to a 1 mL Dounce homogenizer and homogenize thoroughly.
- Mix the homogenate on an orbital shaker at room temperature for three to four hours. The sample is stable at this stage, and can undergo multiple freeze-thawed cycles.
- Dilute the sample ten-fold with cold PBS with 1X protease inhibitor cocktail. Centrifuge at 16,000 × g for 20 minutes at 4°C.
- Carefully transfer the supernatant to a new tube, and place it on ice.
- Dilute the tissue extract (supernatant) an additional 1:10 to 1:100 with Standard Diluent Buffer prior to application in the ELISA.