

Sample Collection, Processing, and Storage Guidelines

For ELISA kit

Liquid Samples

- **Serum:** Collect in a sterile tube. Allow the blood to clot naturally for 120 minutes at room temperature or overnight at 2–8°C. Centrifuge at 3,000 rpm for 20 minutes at 2–8°C. Carefully collect the supernatant for immediate assay, or store at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.
- **Plasma:** Choose EDTA or sodium heparin as the anticoagulant according to sample requirements. Mix thoroughly and centrifuge at 3,000 rpm for 20 minutes at 2–8°C within 30 minutes. Carefully collect the supernatant for immediate assay, or store at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.
- **Urine, Pleural/Abdominal Effusion, Cerebrospinal Fluid, and Saliva:** Collect in a sterile tube and centrifuge at 3,000 rpm for 20 minutes at 2–8°C. Carefully collect the supernatant for immediate assay, or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatant:** Collect in a sterile tube and centrifuge at 3,000 rpm for 20 minutes at 2–8°C. Carefully collect the supernatant for immediate assay, or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Solid Samples

- **Animal Tissue Samples:** Rinse tissue with pre-cooled PBS (0.01 M, pH = 7.4) to remove residual blood. Weigh and mince tissue into small pieces. Transfer the minced tissue into a glass homogenizer with an appropriate volume of PBS (typically a 1:9 w/v ratio, e.g., 1 g tissue to 9 mL PBS; adjust as needed and record the volume; adding protease inhibitors to the PBS is recommended). Grind thoroughly on ice/at low temperature, or use a tissue grinder. If further cell lysis is needed, perform sonication on the homogenate. Finally, centrifuge the homogenate at $5,000 \times g$ for 10 minutes at 2–8°C and collect the supernatant for assay. For other tough tissues, grind thoroughly in liquid nitrogen if glass homogenizers or tissue grinders prove insufficient.
- **Plant Tissue Samples:** Rinse tissue with pre-cooled PBS (0.01 M, pH = 7.4) to remove residual soil and pesticides. Weigh and mince tissue into small pieces. Transfer the minced tissue into a glass homogenizer with an appropriate volume of PBS (typically a 1:9 w/v ratio, e.g., 1 g tissue to 9 mL PBS; adjust as needed and record the volume; adding protease inhibitors to the PBS is recommended). Grind thoroughly on ice/at low temperature, or use a tissue grinder. If further cell lysis is needed, perform sonication on the homogenate. Finally, centrifuge the homogenate at $5,000 \times g$ for 10 minutes at 2–8°C and collect the supernatant. Aliquot the supernatant for single use, store at -20°C or -80°C, and avoid repeated freeze-thaw cycles.

Cell Samples

- **Animal Cells:** For adherent cells, wash gently with pre-cooled PBS and detach using trypsin. Collect cells by centrifugation at $1,000 \times g$ for 5 minutes (suspension cells can be collected directly by centrifugation). Discard supernatant and wash cells 3 times with cold PBS. Resuspend cells in cold PBS at a density of 1×10^7 cells/mL. Disrupt cells thoroughly using a sonicator to release intracellular components. Centrifuge at 3,000 rpm for 20 minutes at 2–8°C, then carefully collect the supernatant for assay.
- **Plant Cells:** Dilute the cell suspension with PBS (pH 7.2–7.4) to reach approximately 1×10^6 cells/mL. Place on ice and disrupt cells thoroughly using a sonicator. Centrifuge at 3,000 rpm for 20 minutes at 2–8°C, and carefully collect the supernatant for assay.

Throat Swabs

Add 2 mL of PBS (pH 7.2–7.4) to dissolve the swab head and shake well. Remove the swab with forceps, squeezing out remaining liquid. Centrifuge at 2,000–3,000 rpm for approximately 20 minutes at 2–8°C, then carefully collect the supernatant. Keep one aliquot for testing and freeze the remainder for future use. If precipitation forms during storage, re-centrifuge prior to loading.



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Sample Requirements

Perform extraction as soon as possible after sample collection, and run assays promptly following extraction. If assays cannot be run immediately, store samples at 2–8°C for use within 6 days. Otherwise, store at -20°C (≤ 1 month) or -80°C (≤ 2 months). Avoid repeated freeze-thaw cycles. If precipitation forms during storage, re-centrifuge prior to loading. Samples containing NaN₃ cannot be analyzed, as NaN₃ inhibits Horseradish Peroxidase (HRP) activity.