Technical Manual

Lysis Buffer (ELISA Tissue/Cell Processing Buffer)

- Catalogue Code: LBFI0001
- Research Use Only

1. Product Description

The Assay Genie Lysis Buffer is a rapid and efficient solution for lysing tissue and cell samples. Protein extracts prepared using this buffer are fully compatible with downstream applications such as ELISA.

This reagent is suitable for animal cells, or tissue samples. Its primary components include: 50 mM Tris (pH 7.2–7.4), 0.9% NaCl, 0.1% SDS. Users may optionally add specific inhibitors depending on their experimental requirements. To measure protein concentration in lysates prepared with the Assay Genie Lysis Buffer, we recommend using the BCA Protein Assay Kit. Please note that due to the high detergent content, the Bradford assay is not suitable for quantifying these samples.

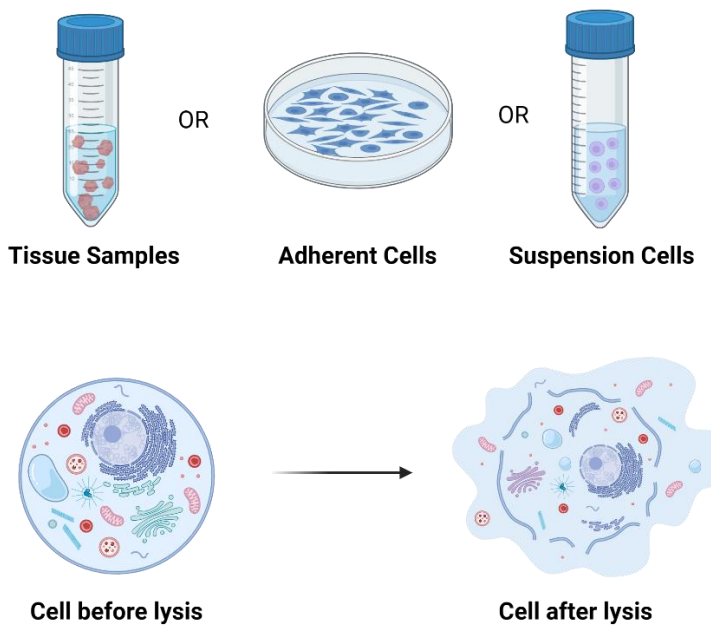

Figure 1. Schematic representation of sample treatment with Lysis Buffer (ELISA Tissue/Cell Processing Buffer) LBFI0001. The Lysis buffer reacts and destroys the cell membrane. As a consequence, intracellular components are released (i.e., proteins, lipids, nucleic acids, reporter molecules, cell signal molecules and other small biomolecules).

2. Kit components

Item	Description	Size
1	Lysis Buffer (50mM Tris,0.9% NaCL,0.1% SDS, pH 7.3)	100 ml (liquid)

3. Storage and expiry date

- **Storage Conditions:** -20°C for 12 months. For regular use, it can be stored at 2-8°C for a short time.
- **Expiration Date:** The expiration date of the kit is clearly indicated on the **outer packaging box**. Do not use the kit beyond this date.

4. Application Method

A. Tissue Sample Preparation (Homogenates)

To prepare tissue homogenates for downstream applications such as ELISA, follow the detailed steps below:

1. **Dissection and Storage**: Use clean, sterile tools to dissect the target tissue and immediately place it on ice to prevent protease degradation. For tissues not processed immediately, transfer them into microcentrifuge tubes, snap-freeze in liquid nitrogen, and store at –80°C for later use.
2. **Washing and Pre-treatment**: Rinse tissues with ice-cold PBS buffer (0.01 M, pH 7.4) to remove residual blood. Weigh the tissue and, if necessary, cut larger pieces into smaller fragments for easier homogenization.
3. **Homogenization**: Prepare the grinding buffer on ice. Typically, use approximately 9 mL of **Lysis buffer** per 1 g of tissue. Add protease inhibitors such as 1 mM PMSF as needed. Homogenization can be performed using ultrasonic disruption (with ice bath cooling) or by applying two freeze-thaw cycles.
4. **Centrifugation**: Centrifuge the homogenate at 5000 × g for 5 minutes. Collect the supernatant for immediate use in assays or aliquot and store at –20°C or –80°C for long-term storage.

5. **Protein Quantification (Recommended):** If required, determine the total protein concentration to support statistical analysis or normalization. Typical protein concentrations fall between 1–3 mg/mL. ***Note:*** *Tissue types such as liver, kidney, and pancreas often contain high levels of endogenous peroxidase, which can react with TMB substrate and result in false positive signals in ELISA assays.*

B. Cell Lysate

Suspension Cells

1. **Cell collection:** Centrifuge the suspension cells at 2500 rpm at 2–8°C for 5 minutes and collect the cell pellet.
2. **Washing:** Resuspend the collected cells in pre-chilled PBS, gently mix, then centrifuge again at 2500 rpm at 2–8°C for 5 minutes. Collect the cell pellet.
3. **Lysis:** Add 0.5–1 mL of **Lysis Buffer**. Add protease inhibitors, such as PMSF, to a working concentration of 1 mM. Avoid using buffers containing NP-40, Triton X-100, or high-concentration DTT, as they can interfere with antigen-antibody reactions. Lyse the cells on ice for 30 minutes to 1 hour. During lysis, pipette up and down or gently shake intermittently to ensure complete protein extraction. If viscous, due to sticky DNA, apply ultrasonic disruption (recommended ultrasonic conditions (on ice): 3–5 mm probe, 150–300 W, 3–5 seconds per burst, with 30-second intervals, for a total working time of 1–2 seconds).
4. **Centrifugation:** After lysis or ultrasonic disruption, centrifuge at 10,000 rpm at 2–8°C for 10 minutes.
5. **Storage & Use:** Carefully collect the supernatant and transfer it to an microcentrifuge tube. Use the lysate immediately for assays or aliquot and store at –80°C for future use.

Adherent Cells

1. **Washing:** Remove the culture medium and wash the cells three times with ice-cold PBS.
2. **Lysis:** Add 0.5–1 mL of **Lysis Buffer**, plus protease inhibitors (e.g., PMSF at 1 mM final concentration, as in suspension cell protocol). Gently scrape the cells with a cell scraper.
3. **Collection and Disruption:** Transfer the scraped cells into a centrifuge tube. Lyse on ice for 30 minutes to 1 hour or disrupt using ultrasonic conditions as described for suspension cells.
4. **Centrifugation:** Centrifuge the lysed sample at 10,000 rpm for 10 minutes. Collect the supernatant carefully.
5. **Storage or Use:** Transfer the supernatant to an EP tube. Use immediately for assays or aliquot and store at –80°C.

Additional Notes:

- **Ultrasonic Disruption:** Recommended for breaking down DNA during lysate preparation; fragmented DNA does not significantly affect ELISA performance.
- **Sample Type Selection:**
    - For secreted targets (e.g., secreted or membrane-bound proteins), use the cell culture supernatant.
    - For intracellular targets, use the cell lysate. Be aware that some intracellular proteins may leak into the medium due to secretion or apoptosis, making the supernatant detectable as well.

5. Precautions

- **PMSF:** Please prepare PMSF freshly and add it to a final concentration of 1 mM just before use. Alternatively, you may add a suitable mixture of protease and phosphatase inhibitors as needed.
- **Temperature:** Perform all lysis steps on ice or at 4°C to maintain protein integrity.
- **Intended Use:** This product is intended for research use only. It is not for use in clinical diagnostics, treatments, food, or pharmaceuticals, nor should it be stored in household settings.

- **Safety Precautions**: Always wear a lab coat and disposable gloves when handling this product to ensure your safety.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@AssayGenie.com
Web: www.AssayGenie.com