Lysis Buffer (for ELISA)

Cat #: orb2282947 (manual)

For Research Use Only.

Product Features

Size: 100 ml

Form: liquid

Reagent composition:50mM Tris, 0.9%NaCL, 0.1%SDS, pH7.3

Application: Lysate of tissue samples or cell samples

Storage: Store at -20°C, valid for one year. When used frequently, it can be stored at 2-8°C for a short time.

(At low temperature, the reagent crystallizes. Before use, it needs to be balanced at room temperature or 37°C for 30min to completely dissolve.)

Method of Application

A. Tissue Samples

Tissue samples are processed into tissue homogenates. Detailed steps are listed below:

1) Use a clean tool to dissect the target tissue and quickly put it on the ice to prevent protease from degradation. (Put the temporarily unused tissue into circular microcentrifuge tube and rapidly immerse the tube into liquid nitrogen for cryopreservation. Store the sample at -80°C for next time usage.)

2) Wash tissues with pre-cooling PBS buffer (0.01M, pH=7.4) to remove residual blood. Then weigh for usage. Cut larger tissues into fragments first.

3) Use the grinding buffer on the ice. PBS buffer is commonly used. It's suggested to integrally use **Tissue/Cell Processing Buffer (**50mM Tris+0.9%NaCL+0.1%SDS, pH7.3) or RIPA lysis buffer(medium). Notes: RIPA lysis buffer requires for pH7.3. Components containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentration are not recommended due to the interfering with antigen-antibody reaction. Grinding tissue homogenate: The volume of added grinding buffer depends on the tissue weight. Usually, 9ml grinding buffer is appropriate for 1g tissue fragment. Add some protease inhibitors (e.g. 1mM PMSF) into PBS buffer. The processed homogenate can be subject to ultrasonic disruption or free-thaw cycles. (Ice bath cooling is required for ultrasonic disruption. Free-thaw cycles can be repeated twice.);

4) Centrifuge the processed homogenate for 5 minutes at 5000×g. Then collect the supernatant to perform the assay or sub-pack for cryopreservation at -20°C or -80°C.

5) Tissue homogenate samples can quantify the total protein to get statistical analysis data according to requirements of the assay. Usually, the concentration of total protein is within 1-3mg/ml. Some tissue samples (e.g. liver, kidney, pancreas) containing endogenous peroxygenase with higher concentration may react with TMB substrate, and then false positive appears.

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B. Cell Lysate

(1) Suspension Cell

1.1. Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect the cell.

1.2. Add pre-cooling PBS into collected cell and gently mix; Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect the cell.

1.3. Add 0.5-1ml **Tissue/Cell Processing Buffer (**50mM Tris+0.9%NaCL+0.1%SDS, pH7.3), then add some protease inhibitors (e.g. PMSF, working concentration: 1mmol/L). Alternatively, add RIPA lysis buffer (medium). RIPA lysis buffer requires for pH7.3. Components containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentration are not recommended due to the interfering with antigen-antibody reaction. Lyse the cell on the ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. If sticky DNA appears, ultrasound can disrupt DNA. (Another ultrasonic condition for processing sample on the ice: 3-5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

1.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into an EP tube. Perform the assay immediately or sub-pack for cryopreservation at -80℃.

(2) Adherent Cell

1.1. Absorb supernatant and add pre-cooling PBS to wash three times.

1.2. Add 0.5-1ml **Tissue/Cell Processing Buffer (**50mM Tris+0.9%NaCL+0.1%SDS, pH7.3), then add some protease inhibitors (e.g. PMSF, working concentration: 1mmol/L)(read requirements in suspension cell). Scrape adherent cell gently with a cell scraper.

1.3. Add the cell suspension into centrifugal tube. Lyse the cell on the ice for 30min-1h. Or disrupt the cell by ultrasound(read requirements in suspension cell).

1.4. At the end of lysate/ultrasonic disruption, centrifuge at 10000rpm for 10 minutes. Then, the supernatant is added into EP tube. Perform the assay immediately or sub-pack for cryopreservation at -80℃.

Notes:

It's recommended to use ultrasound to disrupt during cell lysate preparation. Ultrasound can break the DNA efficiently. DNA fragments won't greatly interfere with the performance of the ELISA kit.

Selection of Cell Culture Supernatant and Cell Lysate

Select cell lysate or cell culture supernatant as the sample according to the position of target object.

If the target object is secreted (e.g. secreted membrane protein), cell culture supernatant can be used as the sample.

It's recommended to select cell lysate for intracellular target object. Some intracellular proteins may leak into the medium by secretion or apoptosis. However, the supernatant is still detectable.