ELISA LYSIS AND PROTEIN EXTRACTION BUFFER

Storage: 4°C
Safety: Avoid eye and skin contact and ingestion.

Chemical Properties of ELISA Protein Extraction Buffer

The ELISA protein extraction buffer contains a mild detergent enabling dissolution of cell membranes, solubilization of most membrane proteins and suspension of cytosolic proteins into the buffer.

Contents

1. 60 ml ELISA protein extraction buffer solution; 1X

Protocol Overview: ELISA Protein Extraction Buffer

1. Prepare 500 µl ELISA protein extraction buffer for each 10-cm dish, or 200 µl for each well in a 6-well dish. Make the solution ice cold and add protease inhibitors, including serine protease inhibitor (such as PMSF). If needed for the particular antigen, also add general phosphatase inhibitor.

2. Remove media and rinse cells three times with PBS.

3. Add 500 µl ice cold ELISA Protein Extraction Buffer with protease/phosphatase inhibitors into each 10-cm dish. Rotate the dish to cover cells with extraction buffer and place on a bed of ice for 5 min.

4. Scrape cells off the dish, and using a plastic transfer pipette, transfer the cell suspension into an appropriate tube. Rotate and tap the tube several times to dissolve cell membranes. You should observe fibrous materials which correspond to genomic DNA, an indicator of cell lysis. If fibrous materials are not apparent, add 20 µl aliquots of ELISA protein extraction buffer, and rotate and tap the tube until the fibrous materials are observed. Keep the tube on ice for another 15 min.

5. Centrifuge the tube for 15 min at highest speed in a refrigerated microcentrifuge. Collect the supernatant, which will be used in the ELISA assay. A protein concentration assay can be used to quantify total protein concentration. The supernatant can be stored at -80°C long term.

6. For ELISA assays, dilute the supernatant in sample diluent to meet the concentration range of the assay.

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