Tissue and Cell Lysate Preparation Protocols

Tissue Lysis Buffer:

200 mM NaCl, 10 mM Tris-HCl pH 7.4, 5 mM EDTA, 1% Nonidet P-40 or Triton* X-100, 10% glycerol, 1 mM oxidized L-glutathion, 100 µM PMSF, 2.1 µM leupeptin and 0.15 µM aprotinin
IMPORTANT: PMSF should be freshly added right before use.

Storage: -20 C.

Application:

For preparing tissue or cell lysates for GeniePlex immunoassays by following the procedures described below. Refer to the GeniePlex Multiplex Immunoassay User Manual and kit inserts for the assay procedure after the lysates are prepared.

Preparing Lysate from Adherent Cells:

1. Seed, grow and treat cells under appropriate experimental conditions.
2. Remove culture medium by aspiration.
3. Wash once with ice cold 1x PBS (Optional).
4. Add 200 µL of ice cold lysis buffer/well of a 24-well plate.
5. Pipette up and down 10-12 times.
6. Incubate on ice for 5 min.
7. Centrifuge extract at 14,000xg for 10 minutes at 2-8˚C.
8. Collect the supernatant fraction for GeniePlex assays.

Preparing Lysate from Non-Adherent Cells:

1. Seed, grow and treat cells under appropriate experimental conditions.
2. Pellet cells by centrifugation (300xg, 5 minutes at 2-8˚C).
3. Wash once with ice cold 1x PBS (Optional).
4. Add 500 µL of ice cold lysis buffer/10^7 cells.
5. Pipette up and down 10-12 times.
6. Incubate on ice for 5 min.
7. Centrifuge extract at 14,000 x g for 10 minutes at 2-8˚C.
8. Collect the supernatant fraction for GeniePlex assays.

Preparing Tissue Lysate:

1. Add 500 µL of ice cold lysis buffer/100 mg of tissue.
2. Homogenize at 2-8˚C by using a tissue homogenizer such as POLYTRON* PT1300D or QIAGEN's TissueLyser*.
3. Incubate on ice for 5 min.
4. Centrifuge extract at 14,000 x g for 10 minutes at 2-8˚C. 5. Collect the supernatant fraction for GeniePlex assays.

Note: If measuring protein concentration in the lysate is needed, Lowry Protein Assay is recommended because the lysis buffer contains Nonidet P-40 or Triton* X-100

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