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NBP2-22129 Protocol

Pathway Assay Lysis Buffer Protocol

Pathway Assay Lysis Buffer - Catalog # NBP2-22129

Contents
6x 1.8 ml vials of dried 10Xconcentrated buffer (Store at -20C)
1x 60 ml bottle of 1X Buffer Diluent (Store at 4C)
1x Product sheet

Intended Use
Pathway Assay Lysis Buffer (Catalog # NBP2-22129) at 1X concentration is intended to be used as the direct cell/tissue lysis buffer for samples preparation towards use in ELISA assay with our Pathway Assay Kits. This buffer can be used directly on washed fresh cell cultures as well as excised tissues. It contains powerful denaturing agents, non-ionic detergents, and proteinase and phosphatase inhibitors that have been specially prepared to give optimal performance in our ELISA based Pathway Assay Kits.

Reconstitution Instructions:
1. Each vial of dried 10X concentrated buffer must be reconstituted with 1 ml of the 1X Buffer Diluent to give a final 1 ml of 10X lysis buffer concentrate.
2. The reconstituted 10X concentrate can then be diluted further with the 1X Buffer Diluent (1 part 10X buffer to 9 parts 1X Buffer Diluent) to give up to 10 ml/vial of 1X lysis buffer.

Note: It is important to only use our Buffer Diluent for reconstitution as this will ensure the correct urea and Triton X-100 concentrations in the final working buffer. When stored at -20C, reconstituted buffer would be good for up to 3 months. It is recommended to only make as much 1X buffer as is needed for weekly use. Avoid multiple freeze thaw cycles.

The working concentration Pathway Assay Lysis Buffer contains:
10 mM Tris, pH 7.4
150 mM NaCl
1 mM EDTA
1 mM EGTA
0.5% (v/v) Triton X-100
6M Urea
2 ug/ml Leupeptin
10 uM Pepstatin
3 ug /ml Aprotonin
1 mM Sodium Orthovanadate (Na4VO4)
2 mM Sodium Pyrophosphate (Na4P2O7)
5mM Sodium fluoride (NaF)

Recommended Use amounts:
Note: The researcher is recommended to optimize the cell extraction procedures for their own applications. The instructions below are provided as a general guideline only.

0.5 ml/ 2 million cells
10 cm plate, 70-80% confluent: 0.5 ml Pathway Assay Lysis Buffer per plate.
60 cm plate, 70-80% confluent: 0.3 ml Pathway Assay Lysis Buffer plate.
6 well plate, 70-80% confluent: 0.3 ml Pathway Assay Lysis Buffer per well.
24 well plate, 70-80% confluent: 0.15-0.2 ml Pathway Assay Lysis Buffer per well.
~5 mg piece of tissue, add approximately 0.3 ml of Pathway Assay Lysis Buffer.

Suggested protocol:

Cell Lysate Preparation (adherent cells):
1. Aspirate media and treat the cells by adding fresh media containing regulator for the desired time.

2. Rinse once or twice with ice cold PBS on ice.
3. Remove PBS and add 0.5 ml of Pathway Assay Lysis Buffer (Catalog # NBP2-22129) containing protease/phosphatase inhibitors to each plate (10cm diameter, 70% - 80% confluent). Incubate for 10 minutes on ice. Smaller wells will require less buffer (i.e. for 80% confluent 24 well samples use 150-200 microliters per well).
4. Gently scrape cells off the plate and transfer to chilled tubes/vials. Keep on ice.
5. Vortex briefly and microcentrifuge at 14,000 rpm for 10 minutes at 4C.
(Note: the resulting supernatant should be clear and in a liquid state, not gel like. If it the solution is gel like then too many cells may have been lysed initially. Add another 0.5 ml of lysis buffer; let it sit on ice 5 minutes, repeat vortexing and microcentrifugation step).
6. Aliquot the clear lysate to clean and chilled tubes. These samples are ready for total protein concentration determination and testing. Lysates can be stored at -80 degrees C. Multiple freeze/thaw cycles must be avoided.

Lysate Preparation (Tissues)
1. Our Pathway Assay Lysis Buffer (Catalog # NBP2-22129) buffer may be used for lysing most of the tissues types. Brain, kidney and liver tissue are fairly easy to lyse while heart and muscle tissues may need to be first ground in liquid nitrogen or sheared mechanically prior to being added into Pathway Assay Lysis Buffer. Sonication works well to break up the initial tissues/cells, however, mini-pestles may also be employed.
2. The amount of Pathway Assay Lysis Buffer to use will depend upon the amount as well as type of tissue and for 5mg of tissue, we generally recommend 300ul of Pathway Assay Lysis Buffer (Catalog # NBP2-22129) as a starting point. After sonication or disruption, the goal should be to have relatively clear solution and if there are pieces and/or if it is thick then more lysis buffer may be added.
3. The lysed homogenate should be incubated on ice for about 10 minutes. Afterwards, vortex briefly and microcentrifuge at 14,000 rpm for 10 minutes at 4C. (Note: the resulting supernatant should be clear and in a liquid state, not gel like. If the solution is gel like then add another 0.5 ml of lysis buffer, incubate it on ice for another 5 minutes, repeat vortexing and microcentrifugation step).
4. Aliquot the clear lysate to clean and chilled tubes. These samples are ready for total protein concentration determination and testing. Lysates can be stored at -80C. Multiple freeze/thaw cycles must be avoided.

Note: Because the tissues contains multiple type of cells and not all cell types may express the target under investigation (whereas in cell culture, it is more of a synchronized cell population each expressing or not, a given target to almost same level), it may be required to use more total protein extract than if using a synchronized cell population from cultured cells to get an appropriate signal to noise.