Translocation Assay Protocol

The following protocol is for a 10 cm cell culture dish of confluent cells with 10 mls of media. Adjust volumes of reagents used in the protocol accordingly to the surface area of other sized cell culture dishes.

1. Culture cells in a 10 cm cell culture dish until 80-90% confluency. Set aside an equivalent number of cell culture plates for experimental and control treatments. You may wish to set up additional plates of cells to extract total cell lysates with a cell lysis buffer, such as RIPA buffer, for comparisons to the fractionated materials in downstream Western blots.

2. Prepare the following reagents: PBS at 37 degrees C (not provided); and Cytoplasmic Protein Extraction Buffer (CPEB-1), Wash Nuclear Pellet Buffer-1 (WNPB-1), Nuclear Protein Extraction Buffer (NPEB-1) and 1M DTT on ice. Immediately prior to use, add a general protease inhibitor cocktail to the three buffers. Also, immediately prior to use, add DTT to the Cytoplasmic Protein Extraction Reagent to make a final concentration of 1 mM DTT.

3. Briefly wash cells with PBS warmed to 37^{o}C, remove all PBS and add 500 microliter of Cytoplasmic Protein Extraction Buffer (CPEB-1) for 10 min with the cell culture dish placed on a bed of ice.

4. Scrape and collect the cells in a 1.5 ml microcentrifuge tube.

5. Centrifuge in a refrigerated microcentrifuge (4 degrees C) at 1000 x g for 5 min. Collect the supernatant, which corresponds to the cytoplasmic fraction.

6. Wash the pellet by adding 500 microliters of Wash Nuclear Pellet Buffer (WNPB-1), gently tap tube to mix and then centrifuge again at 1000 x g for 5 min at 4 degrees C. Remove all of the supernatant.

7. Add 60 microliters of Nuclear Protein Extract Buffer (NPEB-1) to the pellet for 10 min on ice. Vortex for 1 min full speed. Centrifuge at 16,000 x g for 10 min at 4 degrees C. Remove the supernatant, which contains the extracted nuclear protein.

8. Resolve proteins in SDS-PAGE gels and Western blots. It is recommended that adjoining lanes in the gel are loaded with total cell lysate, cytoplasmic fraction and nuclear fraction. Since the proteins in the cytoplasmic and nuclear fractions are concentrated, it is recommended that samples are diluted in Laemmli Sample buffer in an empirically determined dilution series to preclude saturation of the corresponding protein bands on Western blots. A 1:1000 dilution of the nuclear fraction sample in Laemmli sample buffer is a recommended dilution that should be included in the analysis.