# Nuclear Protein Isolation-Translocation Assay Kit

Part No. NPI-1

**Assay Capacity**
Suitable for one hundred 10 cm round culture dishes with semi-confluent (80-90%) cells.
Scalable to three hundred wells in six-well dishes with semi-confluent (80-90%) cells.

**Safety Precautions:** Although materials in this kit are not considered to be hazardous by OSHA standards, the following precautions are recommended: Avoid skin and eye contact and ingestion. Wear gloves, eye protection and protective clothing while performing this protocol.

**Storage:** -20°C

## OVERVIEW

This nuclear protein isolate kit provides reagents to fractionate cells into cytoplasmic and nuclear fractions. It generates an enriched nuclear fraction containing proteins resident or imported in the nucleus. Transcription factors that have translocated from the cytoplasm to the nucleus are isolated using the procedures in this kit. The nuclear protein isolation kit effectively separates nuclear and cytoplasmic components with minimal cross-contamination.

A table-top microcentrifuge, instead of more specialized equipment such as an ultracentrifuge, is employed to isolate nuclear and cytoplasmic fractions. Cell fractions are partitioned in less than one hour. The isolated cytoplasmic and nuclear fractions can be employed in downstream applications such as EMSA, nuclear protein isolation and transcription factor translocation assays.

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1. Cytoplasmic Isolation Reagent (CIR-1; 110 ml).
2. Nuclear Isolation Reagent (NIR-1; 3.5 ml; 2 vials; or 7 ml in one bottle).
3. DTT (solid). Add 128 µl $dH_20$ to make a 1M solution.

**Materials not provided and required:**

1. Protease inhibitor cocktail, serine protease inhibitor.
2. Cell scrapers.
3. Refrigerated table top microcentrifuge for 1.5 ml tubes.
4. PBS at room temperature.


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**PROTOCOL**

**Overview:** The following protocol is designated for one 10 cm cell culture dish, yet is scalable to other sized or number of cell culture dishes. Adjust reagent volumes accordingly to the surface area of the cell culture dish. The protocol isolates cytoplasmic and nuclear fractions, and concentrates nuclear proteins.

**Cell culture prior to nuclear fraction isolation**
Grow cells until 80-90% confluency prior to performing fractionation.

**Procedures:** All procedures must be performed rapidly without significant delays. Carefully plan out all steps prior to proceeding with the fractionation. A delay between removal of the Cytoplasmic Isolation Reagent in Step 4 and addition of the Nuclear Isolation Reagent in Step 5 may result in suspension and loss of the nuclear protein fraction.

1. *Preparation of Reagents:* Defrost all reagents; place on ice and keep ice cold. Aliquot 1ml Cytoplasmic Isolation Reagent (CIR-1) and 60 μl Nuclear Isolation Reagent (NIR-1) for each 10 cm dish. Add protease inhibitor cocktail and serine protease inhibitor (such as PMSF) to both the Cytoplasmic and Nuclear Isolation Reagents prior to use.

   *Preparation of provided DTT*: Add 128μl $dH_20$ to the provided tube of solid DTT. Vortex until all particulates are suspended. This dilution makes a 1M stock solution. Dilute the 1M DTT solution into the Cytoplasmic Isolation Reagent (CIR-1) to make a final concentration of 1mM DTT immediately prior to use (i.e. add 1 μl DTT solution to 1ml Cytoplasmic Isolation Reagent). Aliquot and store remaining DTT solution at -20$^{o}$C for later use. Keep all solutions on ice while performing the procedures.

2. *Wash cell culture dish:* Remove the cell culture media and gently wash the cell culture dish twice with 10 ml room temperature PBS. Wash gently to limit the dislodging of cells. Tilt the culture dish and use suction and a Pasteur pipette to remove as much PBS as possible. Place the cell culture dish on a bed of ice.

3. *Cytoplasmic Isolation Reagent addition:* Add 500 μl of Cytoplasmic Isolation Reagent (prepared with protease inhibitors and DTT) to the cell culture dish. Rotate the cell culture dish by hand to cover the cells with a film of Cytoplasmic Isolation Reagent. Place the dish on a bed of ice for 5 min.

4. *Cytoplasmic Fraction Isolation:* Using a cell scraper and a plastic Pasteur pipette, remove the cells from the cell culture dish. Dispense the cell suspension into a 1.5 ml microcentrifuge tube. Centrifuge the tube at 2500 rpm for 3 min at 4$^{o}$C.

   Immediately collect the supernatant into a 1.5 ml microcentrifuge tube. This supernatant contains the **cytoplasmic fraction**. Store the cytoplasmic fraction on ice while continuing with the protocol. The cytoplasmic fraction can also be stored at -80$^{o}$C for longer term storage.

   Gently re-suspend the pellet in 500 μl of Cytoplasmic Isolation Reagent (CIR-1) by tapping the tube. Centrifuge the tube again at 2500 rpm for 3 min at 4$^{o}$C. Using a pointed Pasteur pipette, *immediately* remove as much supernatant as possible without disturbing the pellet.

5. *Nuclear Isolation Reagent addition:* Add 60 μl ice cold Nuclear Isolation Reagent (NIR-1) to the pellet. Vortex for 1 min. Place the tube on ice for 10 min.

   Centrifuge at maximum speed for 10 min at 4$^{o}$C using the microcentrifuge. Collect the supernatant fraction which contains the isolated **nuclear fraction**. The nuclear fraction can be stored long term at -80$^{o}$C.

## RESOLVING CYTOPLASMIC AND NUCLEAR FRACTIONS IN WESTERN BLOTS

A dilution series of samples in Laemmli Sample Buffer should be used to empirically determine the optimum concentration of cytoplasmic and nuclear fraction solution to resolve in Western blots. The proteins in both fractions may be present at high concentrations that produce saturated Western blot bands, requiring dilution for later quantitation. The fractionation reagents also contain materials that may interfere with SDS-PAGE resolution, further requiring sample dilution. For example, the nuclear fraction may require a 1/100-1/1000 fold dilution to clearly resolve and quantitate protein bands.

Cell fractionation can be observed in Western blots by resolving a total cellular lysate (without nuclear-cytoplasmic fractionation), followed by consecutive lanes with cytoplasmic and nuclear fractions.

Cytoplasmic and nuclear marker proteins, such as GAPDH and p84, can be employed to calibrate the cell fractionation (see product webpage for example).

**Troubleshooting, Nuclear Protein Isolation:**

1. Cell Fractionation was not observed. Suspension of the nuclear proteins prior to the removal of the cytoplasmic fractionation buffer may have occurred. Resolution: Perform procedures as rapidly as possible without pauses. Verify that protein bands in Western blots are not at saturation densities.
2. The nuclear protein of interest resolved as a smear in a Western Blot. Resolution: Dilute the nuclear protein fraction further in Laemmli Sample Buffer. Heat the sample to 37$^{o}$C for 30 min and vortex for 1 min instead of boiling in Laemmli Sample Buffer.
3. Proteolysis of nuclear proteins was observed. Resolution: Make sure protease inhibitor cocktail and serine protease inhibitor were added immediately prior to performing the protocol.


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