Nuclear/Cytoplasmic Protein Extraction Kit

Catalog#	Description	Pack
SINP001	Cytoplasmic /Nuclear Protein Extraction kit	50X

Introduction:

The nuclei and cytoplasm are currently two hot spots in the fields of cell biology, and high quality of nuclear and cytoplasmic proteins is important for an ideal and reliable results.

Viagene Biotech provides a Nuclear/Cytoplasmic extraction kit formulated for the quick and simple isolation of nuclear and cytoplasmic extracts from mammalian cells or tissue samples in just 1 hour. The extracted proteins are non-denatured native proteins with nuclear protein concentration of about 1.5-4.0μg/μl, which can be used for EMSA (gel mobility shift assays), Western blotting, ELISA, reporter gene detection and enzyme activity determination.

This kit includes enough reagents for performing extraction of 50 samples and will perform as specified if stored as directed and used before the expiration date.

I. Contents of the kit:

Reagents	Pack	Volume
5×Buffer A	1 bottle	35ml
2×Buffer B	1 Vial	1.5ml
Solution I	1 Vial	1.75ml
Solution II	1 Vial	1.75ml
Solution III	1 Vial	1.5ml
PMSF Solution	1 Vial	1.1ml
User Manual	1 set	Set

II. Store Temperature: store the reagents as that indicated on labels.

III. Preparation of working solutions (mark 3 tubes as follows for different solutions, clearly, to avoid mistakes)

A. Make 3ml **Lysis-L:**

Reagents	Volume
5×Buffer A	0.6ml
Solution I	30µl
Solution II	30µl
PMSF Solution*	15µl
dd-H_2O	2.325ml
Total Volume	3.0ml**

Nots：PMSF must be added before Lysis-L used for treating cells.

B. Make 50µl **Extract-H:**

Reagents	Volume
2×Buffer B	25µl
Solution I	0.5µl
Solution II	0.5µl
PMSF Solution*	0.25µl
dd-H_2O	23.75µl
Total Volume	50µl**

Note: * PMSF must be added fresh before use of working solutions.

** The amounts are actual usage for **each** sample extraction. Usually, 10% more than that for each extraction time sample numbers should be prepared.

IV. Nuclear protein extraction:

A. Adherent Cells:

The following procedure can be used for adherent cells grown in a 100 mm dish to 80-90% confluency where 10^7 cells yields ~50µl of nuclear protein and ~200µl of cytoplasmic protein.

1. Keep dishes or flaskets of cell cultures on ice.

2. Discard culture media, add 3ml pre-cooled 1×PBS into dishes or flaskets to rinse cells, and repeat one time.
3. Collect cells into a pre-cooled **1.5ml** microtube by repeat centrifuge at 500xg for 5min at 4°C. Do not harvest cells using proteolytic enzymes; rather use a rubber policeman.
4. Remove PBS and keep cell pellets. Add **1.4ml of Lysis-L** and resuspend the pellets until no mass can be seen. Keep on ice for 10min allowing cells to swell.
5. Centrifuge for 30 seconds (pulse spin) at 4°C, discard the supernatant. Add **200µl of Lysis-L** and resuspend the pellet. Add **4µl of Solution-III** on the inner wall of the tube. Mix the tube up & down for 30sec, then, keep tubes on ice for **3min** to lyse cell membrane. ***This step can be omitted if only nuclear extracts is needed for EMSA.*
6. Pulse spin at 4°C, transfer or aliquot supernatant containing **cytoplasmic proteins** to a new pre-cooled tube. Measure protein concentration and store samples at **-80ºC**. ***This step can be omitted if only nuclear extracts is needed for EMSA.*
7. Add 1.4ml of **Lysis-L** to the tubes with pellet, and tap tubes to resuspend the pellet until no mass can be seen. Add **28µl of Solution-III** on the inner wall of the tube. Mix the tube up & down for 30sec, then, keep tubes on ice for **3-5min** (the lysis of cells could be checked under a microscope).
8. Pulse spin at 4°C and discard supernatant. Pulse spin at 4°C again, remove supernatant as much as possible. **Do not stir the pellets**.
9. Add 50µl of **Extract-H** if the pellet is 1.5-2mm high in a 1.5ml microtube, adjusting volume of **Extract-H** with less or more pellet. Vortex tubes for 15sec at highest setting, then, gently rock the tube on ice for 30 min using a shaking platform, vortex the tubes at highest setting every 5min.
10. Centrifuge at 4℃, 14,000xg for 10min. Transfer or aliquot the supernatant containing **nuclear proteins** to new pre-cooled tubes. Measure protein concentration and store samples at **-80ºC**.

B. Suspension Cells:

The following procedure can be used for a 15 mL cell suspension grown in a T75 flasket where 10^7 cells yields ~50µl of nuclear protein and ~200µl of cytoplasmic proteins.

1. Keep dishes or flaskets of cell cultures on ice.
2. Collect ~10^7 cells into pre-chilled 15 ml tubes. Centrifuge at 500xg for 5min at 4°C, discard culture media. Add 5ml pre-cooled 1×PBS into tubes to rinse cells, centrifuge at 500xg for 5min at 4°C, and discard culture media.
3. Add 3ml pre-cooled 1×PBS and resuspend the pellets. Transfer cell suspension into pre-cooled **1.5ml** microtube(s) by repeat centrifuge at 500xg for 5min at 4°C. Remove PBS and keep cell pellets. Add **1.4ml of Lysis-L** and resuspend the pellets until no mass can be seen. Keep on ice for 10min allowing cells to swell.

4. Centrifuge for 30 seconds (pulse spin) at 4°C, discard the supernatant. Add **200µl of Lysis-L** and resuspend the pellet. Add **4µl of Solution-III** on the inner wall of the tube. Mix the tube up & down for 30sec, then, keep tubes on ice for **3min** to lyse cell membrane. ***This step can be omitted if only nuclear extracts is needed for EMSA.*
5. Pulse spin at 4°C, transfer or aliquot supernatant containing **cytoplasmic proteins** to a new pre-cooled tube. Measure protein concentration and store samples at **-80ºC**. ***This step can be omitted if only nuclear extracts is needed for EMSA.*
6. Add 1.4ml of **Lysis-L** to the tubes with pellet, and tap tubes to resuspend the pellet until no mass can be seen. Add **28µl of Solution-III** on the inner wall of the tube. Mix the tube up & down for 30sec, then, keep tubes on ice for **3-5min** (the lysis of cells could be checked under a microscope).
7. Pulse spin at 4°C and discard supernatant. Pulse spin at 4°C again, remove supernatant as much as possible. **Do not stir the pellets**.
8. Add 50µl of **Extract-H** if the pellet is 1.5-2mm high in a 1.5ml microtube, adjusting volume of **Extract-H** with less or more pellet. Vortex tubes for 15sec at highest setting, then, gently rock the tube on ice for 30 min using a shaking platform, vortex the tubes at highest setting every 5min.
9. Centrifuge at 4℃, 14,000xg for 10min. Transfer or aliquot the supernatant containing **nuclear proteins** to new pre-cooled tubes. Measure protein concentration and store samples at **-80ºC**.

C. Tissue nuclear extraction (Rat liver as an example tissue):

The following procedure is used for 150-200mg rat liver where 10^8 cells yields 50-80µl of nuclear protein and ~200µl of cytoplasmic protein.

1. Take 150-200mg of fresh or frozen rat liver into a microtube. Rinse the tissue with pre-cooled 1× PBS three times.
2. Cut the tissue to very small pieces using a clean razor blade, then, collect the pieces into a pre-chilled, clean Dounce homogenizer.
3. Add the 2ml pre-cooled 1× PBS, Homogenize the sample with a Dounce homogenizer or a polytron device.
4. Filter the grinding mixtures with a 40-100 mesh screen to remove large pieces of tissue and fiber.
5. For the rest of the large tissue, repeat steps 3-4 above one more time.
6. Transfer cell suspension into pre-cooled 1.5ml microtubes by repeat centrifuge at 500xg for 5min at 4°C if the volume of suspension is >2ml.
7. Centrifuge at 500xg for 5min at 4°C, remove PBS and keep cell pellets. Add **1.4ml of Lysis-L** and resuspend the pellets until no mass can be seen. Keep on ice for 10min allowing cells to swell.

8. Centrifuge for 30 seconds (pulse spin) at 4°C, discard the supernatant. Add **200µl of Lysis-L** and resuspend the pellet. Add **4µl of Solution-III** on the inner wall of the tube. Mix the tube up & down for 30sec, then, keep tubes on ice for **3min** to lyse cell membrane. ***This step can be omitted if only nuclear extracts is needed for EMSA.*
9. Pulse spin at 4°C, transfer or aliquot supernatant containing **cytoplasmic proteins** to a new pre-cooled tube. Measure protein concentration and store samples at **-80ºC**. ***This step can be omitted if only nuclear extracts is needed for EMSA.*
10. Add 1.4ml of **Lysis-L** to the tubes with pellet, and tap tubes to resuspend the pellet until no mass can be seen. Add **28µl of Solution-III** on the inner wall of the tube. Mix the tube up & down for 30sec, then, keep tubes on ice for **3-5min** (the lysis of cells could be checked under a microscope).
11. Pulse spin at 4°C and discard supernatant. Pulse spin at 4°C again, remove supernatant as much as possible. **Do not stir the pellets**.
12. Add 50µl of **Extract-H** if the pellet is 1.5-2mm high in a 1.5ml microtube, adjusting volume of **Extract-H** with less or more pellet. Vortex tubes for 15sec at highest setting, then, gently rock the tube on ice for 30 min using a shaking platform, vortex the tubes at highest setting every 5min.
13. Centrifuge at 4℃, 14,000xg for 10min. Transfer or aliquot the supernatant containing **nuclear proteins** to new pre-cooled tubes. Measure protein concentration and store samples at **-80ºC**.

V. Note:

1. Please contact us within 72 hours if the shipping package is broken and/or there is leakage in the package.
2. Please store the reagents at the temperature indicated on the vials or bottles.
3. The entire protocol of extractions should be carried out on ice or at 4°C.
4. The operation in this manual are based on the amounts of cells and tissue be indicated. If increasing volume of cell cultures, users need to prepare more extraction reagents in proportion.
5. The amounts of the nuclear protein obtained from extraction are related to the density and stage of cultured cells harvested, usually, 50µg the nuclear protein can be obtained from 10^7 cells.
6. The concentration of extracted protein can be directly measured by BCA-based kit. For a best and reliable BCA result, suggest using **Extract-H** as a reagent control for nuclear protein, and **Lysis-N** as for cytoplasmic protein. Otherwise, the concentration of proteins may become false high.
7. For safety, lab-coats and gloves should be worn.

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