RayBio® Nuclear Extraction Kit

Catalog #: NE-50

User Manual
August 18, 2016

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RayBio® Nuclear Extraction Kit Protocol

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I. INTRODUCTION

RayBio® Nuclear Extraction Kit provides a simple and efficient way to isolate high quality nuclear and cytoplasmic protein from mammalian cells or fresh tissue with less than 10% contamination between the nuclear and cytoplasm fractions. The extracted proteins meet the requirements for most downstream experiments including Western blot, immuno-precipitation, ELISA, DNA binding assays (such as EMSA), and are ideal for RayBio Transcription Factor ELISAs. By adding two reagents of the RayBio Nuclear Extraction Kit, the cytoplasmic protein is isolated from the cell pellet. After brief centrifugation, cell nuclei are collected and nuclear protein is extracted by adding an additional reagent. The whole procedure can be completed within 2 hours and produces around 1 mg with 2 mg/ml non-denature cytoplasm protein and around 0.5 mg with 5 mg/ml non-denature nuclear protein (numbers from an example 100mm dish of fully confluent HeLa cells, ~8.8 x 10^6).
This kit can process 50 extractions of 10^7 cells each.

II. REAGENTS

	Component	Catalog #	Quantity	Volume / Notes
1	NE Reagent-1	NE-001	1 Bottle	3mL of 10x Solution
2	NE Reagent-2	NE-002	1 Bottle	6mL
3	NE Reagent-3	NE-003	1 Bottle	3mL of 2x Solution
4	Protease Inhibitor Cocktail	AA-PI	5 Vials	Lyophilized powder
5	Phosphatase Inhibitor Cocktail I	AA-PHI-I	5 Vials	60 µl of 100x Solution

III. STORAGE

NE reagent-I, II, and III may be stored for up to 6 months at 2º to 8ºC from the date of shipment. Protease Inhibitor and Phosphatase Set-I should be stored at -20 ºC or -80 ºC (recommended at –80 ºC). Note: the kit can be used within one year if the whole kit is stored at -20 ºC. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1 1x PBS buffer.
2 Precision pipettes to deliver 1 μl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 Tissue homogenizer for tissue samples.
5 Refrigerated centrifuge with swing out rotor and refrigerated microcentrifuge.
6 Other lab consumable materials.

V. SAMPLE PREPARATION

Cell culture preparation:

1. For attached cells, collect cells by trypsin digestion or cell scraper, and centrifuge at 4°C, 300xg for 5 minutes in 15 ml or 50 ml pre-chilled tubes. For suspension cells, collect cells by centrifuge at 4°C, 300g for 5 minutes in 15 ml or 50 ml pre-chilled tubes.
2. Wash cells twice with ice cold 1x PBS by centrifugation at 4°C, 300xg for 5 minutes and then remove any remaining PBS.
3. Determine the corresponding volume of 1x NE Reagent-I, -II, III as indicated in Table 1:

Table 1. Reagent volumes for different cell culture amounts

HeLa cells (full confluency)*	1X NE Reagent-I	1X NE Reagent-II	1X NE Reagent-III
35 mm dish	100µl	25µl	25µl
60mm dish	200µl	50µl	50µl
100mm dish	400µl	100µl	100µl
150mm dish	1000µl	250µl	250µl

*Reagent volumes may need to be adjusted for different cell types.

Tissue preparation

1. Cut tissue into small pieces and wash tissue twice in microcentrifuge tubes with ice cold 1x PBS by centrifugation at 4°C, 300Xg for 5 minutes.
2. Remove any remaining PBS carefully.
3. Determine the corresponding volume of 1x NE Reagent-I, -II, III as indicated in table 2.

Table 2. Reagent volumes for different tissue amounts

Amount of tissue (mg)*	1x NE Reagent-I	1x NE Reagent-II	1x NE Reagent-III
20	200µl	50µl	50µl
40	400µl	100µl	100µl
80	800µl	200µl	200µl
100	1000µl	250µl	250µl

* The density of cells per weight varies depending on the tissue type. More or less volume may be required but should be determined empirically.

VI. REAGENT PREPARATION

1. Preparation of Protease Inhibitor:
   Briefly spin the Protease Inhibitor Vial. Add 60 µl of 1x PBS into the vial to prepare a 100X Protease Inhibitor concentrate. Pipette up and down to mix gently (the concentrate can be stored at -20°C for 5 days). This concentrate will be used in Step 4 and Step 5 (preparation of 1X NE Reagent-I and 1x NE reagent-III

2. Preparation of Phosphatase Inhibitor Set-I:
   Take the Phosphatase Inhibitor Set-I Vial from -20°C to RT and wait the concentrate until thaw. Briefly spin the vial. This 100X concentrate will be used in Step 4 and Step 5 (preparation of 1X NE Reagent-I and 1x NE reagent-III).

3. Preparation of 1x NE Reagent-I:
   Prepare the 1x NE Reagent-I using 10x NE Reagent-I concentrate and Protease Inhibitor concentrate (prepared in Step 1), Phosphatase Inhibitor Set-I (prepared in Step 2)as indicated in Table 3:

   Table 3. Preparation of 1x NE Reagent-I.

Component	**1ml of 1 x NE Reagent-I**	**5ml of 1 x NE Reagent-I**	**20ml of 1 x NE Reagent-I**
10 x NE Reagent-I	100μl	500μl	2000μl
Protease Inhibitor	10μl	50μl	200μl
Phosphtase Inhibitor Set-I	10μl	50μl	200μl
Distilled Water	880μl	4400μl	17600μl
Total volume	1ml	5ml	20ml

   Please note: the 1x NE Reagent-I should be made fresh each time and kept on ice during the experiment. Any leftover 1x NE Reagent-I should be discarded.

4. Preparation of 1x NE Reagent-III:
   Prepare the 1x NE Reagent-III using 2x NE Reagent-III concentrate and Protease Inhibitor concentrate (prepared in Step 1), Phosphatase Inhibitor Set-I (prepared in Step 2) as indicated in Table 4:

Table 4. Preparation of 1x NE Reagent-III

Component	**0.25ml of 1 x NE Reagent-III**	**1.25ml of 1 x NE Reagent-III**	**5ml of 1 x NE Reagent-III**
2 x NE Reagent-III	125µl	625µl	2500µl
Protease Inhibitor	2.5µl	12.5µl	50µl
Phosphatase Inhibitor Set-I	2.5µl	12.5µl	50µl
Distilled Water	120µl	600µl	2400µl
Total volume	250µl	1250µl	5000µl

Please note: The 1x NE Reagent-III should be made freshly and kept on ice during experiment. Any leftover 1x NE Reagent-III should be discarded.

VII. EXTRACTION PROCEDURE:

1. Add appropriate volume of 1x NE Reagent-I based on Table 1 or Table 2 to cell cultures or tissues washed with cold PBS as described above in Section V. For cell cultures, pipet up and down gently to disperse the cells. For tissues, homogenize tissue using a Dounce homogenizer or tissue grinder. Transfer cell suspension into 1.5 ml microcentrifuge tubes.

2. Put tubes on ice and incubate cells for 15 minutes.

3. Add appropriate volume of 1x NE Reagent-II based on Table 1 or Table 2 to tubes containing cell suspension and mix gently. Incubate cells on ice for 2 minutes.

4. Centrifuge tubes at 4°C, 14000 x g for 30 seconds in microcentrifuge. Transfer supernatant into new tubes and label it as cytoplasm fraction and store at -80°C.

5. Add appropriate volume of 1x NE Reagent-III based on Table 1 or Table 2 to the tubes containing pellets and suspend pellets completely by vortexing on highest setting for 10 seconds.

6. Put tubes on ice and vortex for 10 seconds every 10 minutes for total incubation of 40 minutes.

7. Centrifuge tubes at 4°C, 14000 x g for 10 minutes in microcentrifuge. Transfer supernatant and aliquot into new tubes. Label it as nuclear fraction and store at -80°C until use.

VIII. TYPICAL DATA

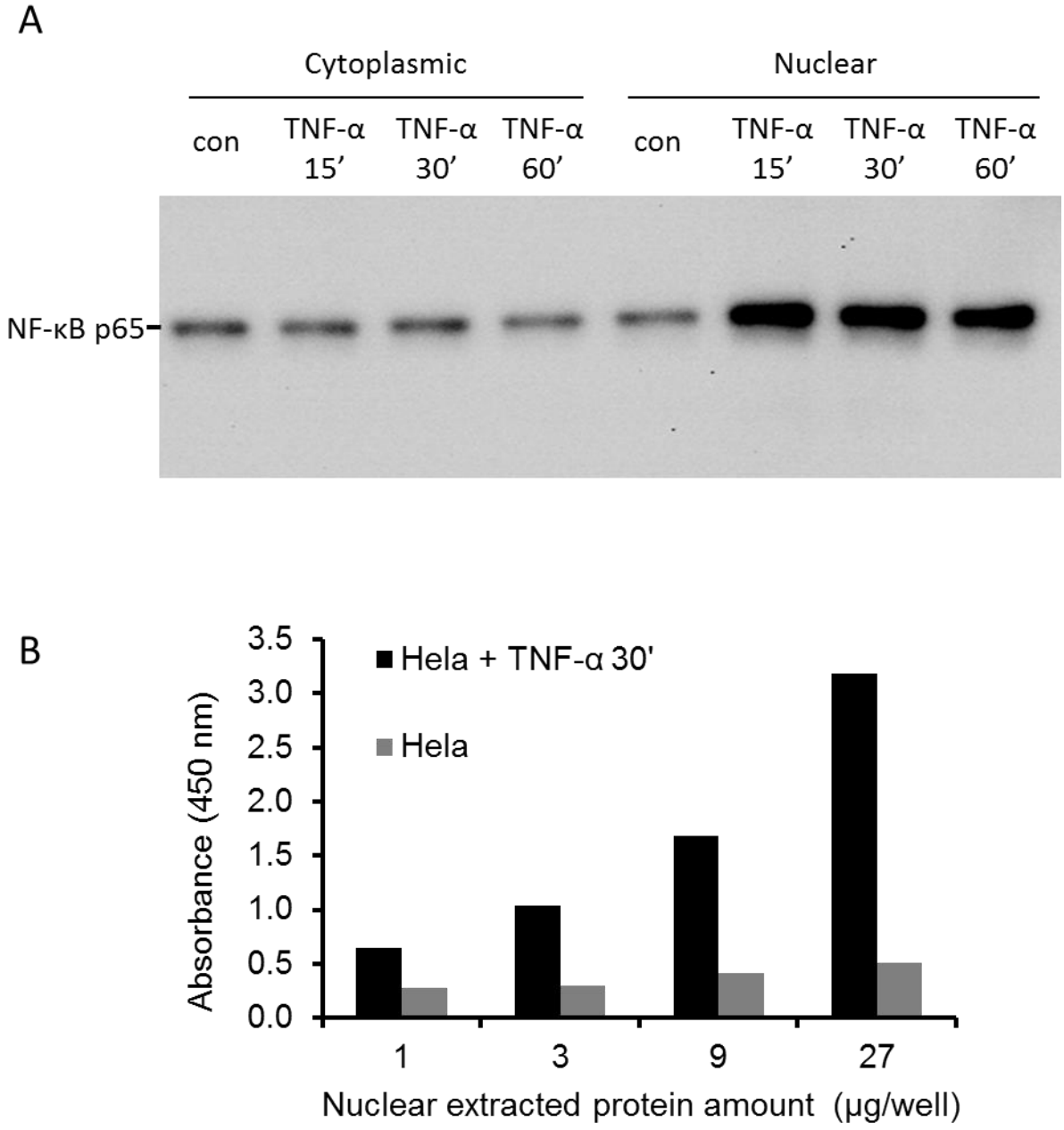

Figure 1. Detection of nuclear fraction of HeLa cell with or without treatment of TNF-α extracted using RayBio Nuclear Extraction Kit. A. Western-blot result of NF-κB p65 from cytoplasm and nuclear fractions. B. Transcription factor assay of NF-κB p65 from nuclear fractions with RayBio NF-κBp65 TF-ELISA Kit (cat # TFEH-p65).

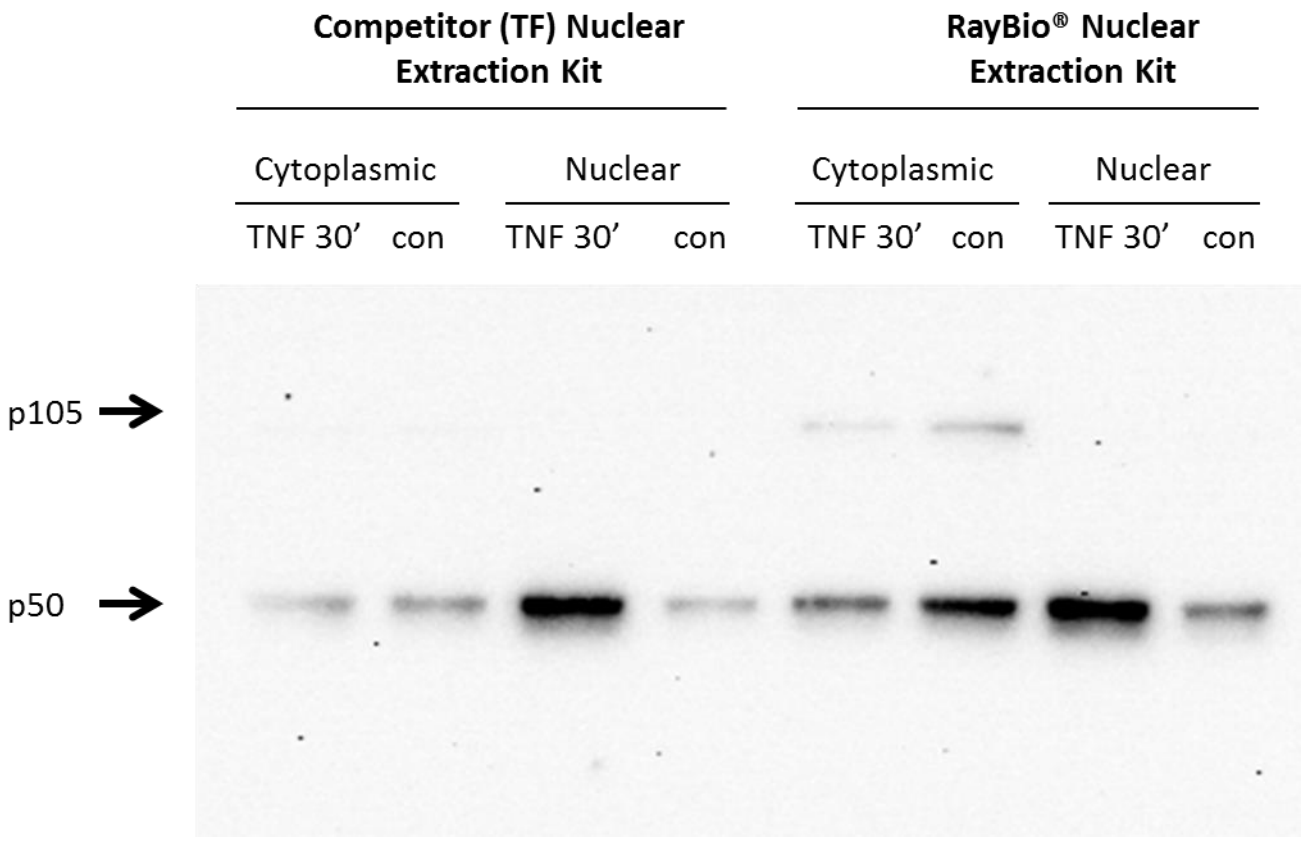

Figure 2. Comparison of RayBio® Nuclear Extraction Kit with a commercial equivalent. NF-κB p65 from cytoplasm and nuclear fractions of HeLa cell with or without treatment of TNF-α is detected by Western blot.

VIII. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Low concentration of proteins in cytoplasm fraction	1.Incorrect volume of NE Reagents	1. Use correct the volume of NE Reagents according to amount of cell cultures or tissues
	2. Cell pellet is not dispersed completely	2. Vortex more or at higher setting.
	3. Tissue is not homogenized thoroughly	3. Homogenize tissue for long enough so that cells get separate completely.
2. Low concentration of proteins in cytoplasm fraction	1. Incorrect volume of NE Reagents	1. Use correct the volume of NE Reagents according to amount of cell cultures or tissues
	2. Cell pellet is not dispersed completely	2. Vortex more or at higher setting during 40 minute incubation.
3. No or low activity of proteins in further assay	Proteins may be degraded during extraction	1. Make sure protease inhibitor and phosphotase inhibitors added in NE Reagents. 2. Make sure to keep sample on ice and spin at refrigerated centrifuge during extraction.

Related Products:

RayBio® Transcription Factor ELISA kits
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