Cytoplasmic & Nuclear Protein Extraction Kit (mammalian cells & tissues)

Cat. #: AMS.P504S (4 rxn); AMS.P504 (20 rxn); AMS.P504L (50 rxn)

Storage: Store at 4°C **Shelf Life:** 12 months

Product Description:

This kit is for fast extraction of **native cytoplasmic and nuclear proteins** from cultured **mammalian cells** or **mammalian tissues**.

The procedure can be completed in less than 1**5 min.**

Extracted native cytoplasmic and nuclear proteins can be used for SDS-PAGE, immunoblottings, ELISA, IP, protein localization, gel mobility shift assays, 2-D gels etc.

This product is for research use only.

Product Components

Components	Amount			Storage
	AMS.P504S	AMS.P504	AMS.P504L	
Cytoplamic extraction buffer	2 mL	10 mL	25 mL	4°C
Nuclear extraction buffer	2 mL	10 mL	25 mL	4°C
Protein extraction filter cartridges	4	20	50	Room temperature
Collection tubes with cap	4	20	50	Room temperature

Additional Materials Required

- 1 X PBS
- Vortexer
- Table-Top Microcentrifuge
- Micro-Tube Pestles (www.rpicorp.com, Cat.# 199221, 199224, 199228 and 299220)

Important Notes:

1. Read the entire protocol carefully.
2. Protease inhibitors is not necessary prior to extraction. However if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, the addition of protease inhibitors to extracted lysate buffer is recommended.
3. The nuclear extraction buffer contains 300 mM salt, for some applications, dilution or desalting of the extract may be needed.
4. The capacity of protein extraction filter cartridge is 500 µL. Multiple filter cartridges can be used if larger amount of cell lysate is processed.

5. It is recommended to use BCA Protein Assay Kit for determination of protein concentration.
6. To study protein phosphorylation, phosphatase inhibitors (such as PhosStop from Roche) should be added to lysis buffer prior to use.

Protocol:

A. From cultured cells in suspension

1. Harvest cells in suspension by low speed centrifugation (**500x g** for **3 min**.).

   Wash the cell once with **cold PBS**.
2. Transfer the cells to a 1.5 mL microcentrifuge tube and pellet the cells by centrifugation at **3,000 rpm** for **1 min**; aspirate the supernatant completely.
3. Add cytoplasmic extraction buffer to cell pellets according to **Table 1**, **vortex** the tube vigorously for 15 seconds, incubate **on ice** for **5 min** and vortex briefly. Go to **step D** – "Cytoplasmic and nuclear protein extraction".

Table 1.

Packed cell volume	Cytoplasmic extraction buffer	Nuclear Extraction Buffer
5 µL	50 µL	25 µL
10 µL	100 µL	50 µL
20 µL	200 µL	100 µL
50 µL	500 µL	250 µL

B. From adherent cells

1. Grow cells to 90-100% confluence and wash the cells **twice** in the tissue culture plates, dishes or flasks with **cold PBS**, and aspirate the buffer **completely**.
2. Add cytoplasmic extraction buffer according to **Table 2**, swirl to distribute the lysis buffer over the entire surface of tissue cultures, place the tissue culture **on ice** for **5 min**. Scrape the lysed cells with a pipette tip or with a transfer pipette. Transfer cell lysate to **pre-chilled** 1.5 mL microcentrifuge tube. **Vortex** the tube vigorously for 15 seconds. Go to **step D** – "Cytoplasmic and nuclear protein extraction".

Table. 2

Containers	Cytoplasmic extraction buffer	Nuclear Extraction Buffer
24-well plate	80 µL	25 µL
6-well plate	300 µL	150 µL
25 cm^2 flask	600 µL	250 µL

C. From tissues

1. Weight desired amount of tissue (5 to 30 mg) and place in a **pre-chilled** 1.5 mL microcentrifuge tube.
2. Wash the tissue once with **cold PBS**. Centrifuge the tissue at **3,000 rpm** for **1 min**; remove supernatant and leave the pellet **as dry as possible**.
3. Add cytoplasmic extraction buffer to the tissue according to **Table 3**. Homogenize the tissue using a micro-tube pestle or a micro-grinder (www.rpicorp.com, Cat.# 199221, 199224, 199228 and 299220). Remove non-homogenized tissue debris. Go to **step D** – "Cytoplasmic and nuclear protein extraction".

Table 3.

Amount of tissues	Cytoplasmic extraction buffer	Nuclear Extraction Buffer
5 mg	50 µL	25 µL
10 ~ 15 mg	100 µL	50 µL
15 ~ 20 mg	200 µL	100 µL
20 ~ 30 mg	500 µL	250 µL

D. Cytoplasmic and nuclear protein extraction

1. Centrifuge the tube for **5 min** at top speed (**14,000 ~ 16,000 rpm**) in a microcentrifuge at **4°C**.
2. Transfer the **supernatant** to a **pre-chilled** 1.5 mL tube. This is your cytoplasmic protein extract.

   (optional: wash the pellet with 0.5 mL cold PBS to reduce contamination of cytosolic proteins.)

   Add appropriate amounts of nuclear extraction buffer to the pellet (according to the tables above), **vortex** vigorously for 15 seconds, incubate the tube **on ice** for **1 min**.

   Repeat 4 times of the following: "vortex for 15 seconds and incubate on ice for 1 min."

3. **Immediately transfer** the nuclear extract mixture to a **pre-chilled filter cartridge** with collection tube and centrifuge at top speed (**14,000 ~ 16,000 rpm**) for **30 seconds**. Discard the filter cartridge according to your institution's waste disposal protocol. The flow through is your nuclear protein extract. The typical protein yield is about 1.5-2.5 mg/ml.
4. Store the cytoplasmic and nuclear protein extract at −80°C until use.

Troubleshooting

Problem	Solution
Low protein concentration	Increase starting cell numbers, or decrease the amount of lysis buffer.
Low protein activity	Keep lysate cold / add protease inhibitors
Significant contamination of nuclear fraction by cytosolic proteins	Add NP-40 to cytosolic extraction buffer to a final concentration of 0.1%

AMSBIO| www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85