

EXOBX® Nucleus Isolation Kit

Cat. no. R6302

Address: SHT technology GmbH, Niederhöchstädter Str. 64, 61476 Kronberg im Taunus, Germany

Tel.: +49 176 34989414

Website: <https://sht-medino.de/>

EXOBX® Nucleus Isolation Kit

Cat. no. R6302

Catalog Number	Reagent In Kit	Size	Storage
R6302	Buffer NA	100 ml	4 °C
	Buffer NB	100 ml	4 °C
	Manual	1	

1. Transportation and Storage

This product is transported at room temperature, stored at 4 °C and valid for 12 months.

2. Important Product Information

- 1) The whole experimental process should be carried out on ice, and the reagents used in the experiment should be precooled.
- 2) To inhibit protein degradation during the procession of nucleus isolation, the protease inhibitors (such as Cocktail) could be added in Buffer NA.
- 3) The concentration of proteins in cytoplasm solution should be measured by using Bradford protein concentration assay method, but not BCA protein concentration assay method.
- 4) Please wear lab clothes, gloves and safety glasses during the experiment.

3. Product Introduction

Nucleus is the largest organelle in the cell, which contains the genetic material, DNA, so it plays a critical role in cell growth and differentiation. The EXOBX®™ Nucleus Isolation Kit for cultured cells enables isolation of intact nucleus from cultured mammalian cells by using sucrose density gradient centrifugation. The isolated nucleus can be used for various subsequent experiments, including transcription regulation assay, protein level and location detection, and even DNA purification.

4. Additional Materials Required

- Phosphate Buffered Saline (PBS)
- RIPA Lysis Buffer (without SDS)
- 1,4-dithiothreitol (DTT)
- Triton X- 100
- Protease inhibitors, EDTA-free such as Protease Inhibitor Cocktail.
- Variable-speed bench-top microcentrifuge (refrigerated)
- Vortex mixer

5. Experimental Procedure

- 1) Reagents Preparation.
 - a) Preparation of Buffer NA working solution. Add DTT to Buffer NA with a final concentration of 1 mM, then add protease inhibitor (such as Cocktail).
 - b) Preparation of cell lysis solution. Add Triton X- 100 to the Buffer NA working solution prepared in the previous step, with the final concentration of 0.2%.
 - c) preparation of Buffer NB working solution. Add DTT to Buffer NB with the final concentration of 1 mM.
- 2) Pellet 2×10^6 cells by centrifuging harvested cell suspension in a 2.0 mL microcentrifuge tube at 1000 g for 3 minutes. Carefully remove and discard the supernatant.
- 3) Add 1 ml cell lysis solution. Vortex at medium speed for 5 seconds to suspend cells and incubate tube on ice for 20 minutes. Mix it upside down every 5 minutes.
- 4) Add 1 ml Buffer NB working solution in new microcentrifuge tubes, and then slowly add the cell lysate from the previous step to Buffer NB working solution along the wall of tubes (note: be sure to add it slowly) . Then, the liquid in the tube is divided into two layers (do not mix it upside down or Vortex, otherwise the experiment will fail).
- 5) Centrifuge at 1,000 rpm (97 g) for 10 min at 4 °C . The liquid is still divided into upper and lower layers.
- 6) Transfer 0.5 ml of the upper layer liquid (do not take the lower layer liquid) to a new microcentrifuge tube, which is the cytosol solution and can be stored at -80 °C .
- 7) Continue to remove the remaining liquid and keep the precipitate, which is the nucleus.
- 8) Add 1 ml Buffer NA working solution, mix it upside down, and resuspend the nucleus.
- 9) Add 1 ml Buffer NB working solution in new microcentrifuge tubes, and then slowly add the nucleus resuspension to Buffer NB working solution along the wall of tubes (note: be sure to add it slowly) . Then, the liquid in the tube is divided into two layers (do not mix it upside down or Vortex, otherwise the experiment will fail).
- 10) Centrifuge at 1 000 rpm (97 g) at 4 °C for 10 min, discard the supernatant, and the precipitate is nucleus.
- 11) Centrifuge at 1 000 rpm (97 g) at 4°C for 2 min, and suck the residual liquid with a pipette.
- 12) The precipitate in the tube is the nucleus, which can be lysed immediately or stored at -80 °C .
- 13) The isolated nucleus could be lysed by using two different reagents or methods.
 - a) Lyse the nucleus by using $2 \times$ Laemmli Buffer, and the lysates could be used for western blot, but not the enzyme activity assay.
 - b) Lyse the nucleus by using RIPA Buffer (without SDS) , and the lysates could be used for both western blot and the enzyme activity assay.

- 1 4) To remove the lysosome, peroxisome and other organelles in cytosol solution, centrifuge the cytosol solution at 12 000 g for 15 min. Transfer the supernatant into new microcentrifuge tubes and discard the precipitate. Mix the cleared cytosol solution with 4 × Laemmli Buffer, boil for 5 min and cool by using ice. The lysate could be used for western blot.

6. Identification of Isolated Nucleus

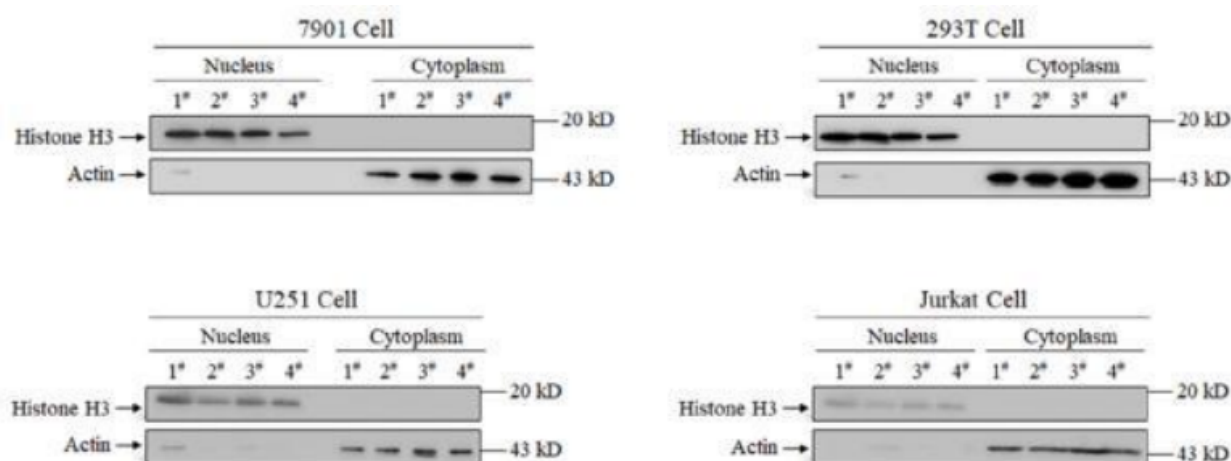


Figure 1. Identification of nucleus isolation

7. Frequently Asked Questions and Analysis

Questions	Reasons	Solutions
The isolated nucleus is less.	Less cells were used to isolate nucleus.	Increase cells for nucleus isolation.
Contamination of cytoplasm or other organelles in purified nucleus	Add cell lysate into Buffer NB working solution, and mix.	Cell lysate should be slowly added to Buffer NB working solution, and do not mix.
	The centrifugation speed in the experiment is high.	Centrifuge at low speed during the procession of nucleus isolation.
The concentration of nucleus protein is low.	Little nucleus was used for lysing.	Increase nucleus for lysing.
	The lysis buffer is weak for lysing nucleus.	Select the lysis buffer with strong protein denaturant, such as 2 × Laemmli Buffer.