Minute™ Detergent-Free Nuclei Isolation Kit

(Non-Sterile)

Catalog number: NI-024

Description

Invent Biotechnologies Minute™ detergent-free nuclei isolation kit is designed to rapidly isolate intact nuclei from animal cultured cells or tissues (fresh or frozen). Unlike many other methods in which detergents are used to lyse the cell membranes, intact nuclei can be isolated from the samples using this patented technology in less than 20 min without using any detergent and a tissue homogenizer. One major problem associates with the use of detergent is the tendency of nuclei aggregation, which is difficult to be separated into single nucleus. To overcome this shortcoming we have developed this detergent-free nuclei isolation kit. The sample is first incubated with buffer A that sensitizes the cells for mechanical disruption. The cell suspension is rapidly passing through a specially designed tilter cartridge. The cell membranes are ruptured when passing through the filter leaving intact native nuclei in the flow through. The nuclei are separated from other small cell debris by low speed centrifugation using a proprietary buffer B.

Application

The native and intact nuclei isolated can be used for a variety of applications which include but not limited to: FACS analysis, chromosome immunoprecipitation (ChIP), immunofluorescence staining, cell cycle analysis and/or apoptosis research. The isolated nuclei can also be used as a starting material for isolation/purification of DNA, RNA, proteins and other cellular components.

Kit components

1. 15 ml buffer A
2. 30 ml buffer B
3. 20 protein extraction filter cartridges
4. 20 collection tubes
5. Plastic rod (2)

Shipping: This kit is shipped at ambient temperature**.**
Storage: Store the kit at 4^{o}C upon arrival**.**

Additional Materials Required

Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 rpm

Important information

This kit, in general, can be used for isolation of nuclei from most animal cultured cells and tissues (fresh or frozen). However the purity and integrity of isolated nuclei are cell/tissue type dependent. A typical yield of intact nuclei from mouse liver or kidney tissue is 1-2 million /20mg. The percentage of isolated nuclei is also cell/tissue type dependent. For most cell and

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tissue types the percentage is between 70-95%. If a fresh tissue yields less than 70% intact nuclei, it is recommended that the tissue is frozen at -20°C for at least 30 min then thaw out on ice. This treatment can increase percentage of intact nuclei for some tissue types. Tissues that have gone through freeze and thaw cycles or have been frozen for a long period of time are expected to have lower yield.

Nuclei Isolation Protocol for Cultured Cells:
(pre-chill buffers on ice)

1. Collect 10-50 million cultured cells by low speed centrifugation (500 X g for 5 min). Wash the cell pellet once with 1 ml cold PBS. Remove the supernatant completely.
2. Resuspend the cell pellet in 500 µl cold buffer A and incubate on ice for 8-10 min. After incubation, vortex the tube vigorously for 20-30 seconds. Transfer the cell suspension to a filter cartridge with collection tube.
3. Centrifuge in a table top microfuge at 14,000 rpm for 20 seconds, resuspend the pellet by pipetting up and down a few times and re-pass the cells through the filter one more time.
5. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 3000 rpm for 2 min. Discard the supernatant.
6. Resuspend the nucleus pellet in 0.5-1.0 ml cold buffer B, centrifuge at 4000 rpm for 8-10 min (this is to remove membrane debris). The pellet contains isolated nuclei.

Nuclei Isolation Protocol for Mammalian Tissues:
(pre-chill buffers on ice)

1. Add 20-40 mg fresh or frozen tissue to the filter cartridge. For frozen tissues, thaw them out completely on ice
2. Add 200 µl cold buffer A to the filter, grind the tissue for about 1-2 min using the plastic rod provided (the plastic rod is reusable, clean by washing with water).
3. Add 300 µl cold buffer A to the same filter, incubate on ice for 5-10 min with cap open. Cap the tube and resuspend the tissue homogenate by inverting the tube a few times.
4. Centrifuge in a table top microfuge at 14,000 rpm for 20 seconds, resuspend the pellet by vortexing and re-pass the cells through the filter one more time.
5. Discard the filter and resuspend the pellet by vortexing vigorously for 10 seconds, centrifuge at 3000 rpm for 2 min. Discard the supernatant.
6. Resuspend the pellet in 0.5-1.0 ml cold buffer B, centrifuge at 4000 rpm for 8-10 min (this is to remove membrane debris). The pellet contains isolated nuclei.

Storage of Isolated Nuclei: Isolated nuclei can be resuspended in tissue a culture medium that contains 5-10% FBS or BSA and stored at 4°C for a few days without significant change in morphology. For long term storage reuspend the nuclei in 0.5 ml buffer B and store at -70-80°C.

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