Bio Basic Inc.

A world leader in serving science

PRODUCT INFORMATION

Nuclear Protein Fractionation Kit

Product information for BSP009:

Introductions:

The Nuclear Protein Fractionation Kit provide an optimized cell extraction buffer, protease inhibitor cocktail, and Phosphatase inhibitor for convenient extraction of nuclear proteins from mammalian tissues and suspension or adherent curture cells. The extracted nuclear proteins can be used for many downstream allpications such as Western blotting, ELISA, 2D electrophoresis, IP, EMSA, Pull down, transcription regulation nuclear enzyme assays. Procedure is simple, no ultracentrifugation and toxic chemicals are involved. The kit is sufficient for $50X10^7$ suspension or adherent curture cells or 50x200mg of mammalian tissues samples.

Compositions:

Components	BSP009
Lysis Buffer	10ml
Hypotonic Buffer	50ml
Phosphatase Inhibitor	300ul
PMSF	600ul
DTT(1M)	60ul

Storage and Transportation:

Transportation at room temperature, after received, store Lysis buffer and Hypotonic Buffer at 4^oC, and keep DTT and Phosphatase inhibitor, PMSF at -20^oC.

Procedures:

1. Wash the cells with ice-cold PBS three times from cell suspension culture. Add ice-cold PBS and scrape the cells off the dish using a cell scraper. Transfer the cells to a pre-chilled centrifuge tube and spin at 600 *g* for 5 minutes at 4°C. Remove supernatant and wash cell pellet by gently resuspending it with ice-cold PBS. Spin at 1000 *g* for 5 minutes at 4°C and remove supernatant, one extraction needs about $1x10^7$ cells.
   Note : For tissue sample ,one extraction need100-200mg ,remove fat and nerve tissue at best, cut it into small pieces, and then wash them with pre-cold PBS for three times.
2. Add 0.6ml ice cold Hypotonic Buffer (before use ,add 1ul DTT and 5ul Phosphatase inhibitor ,10ul PMSF into1ml Hypotonic Buffer), vortex, homogenize them with glass homogenizer for 30-50 strokes or sonicated them for 30 seconds, interval 1minutes,repeat operation for three times. Check the efficiency of cell fracture, must more than 90 percent cells have been broken.
3. Transfer the above homogenized solution into a new pre-cold 1.5ml centrifuge tube , place them on icebag for ten minutes, occasionally vortex for 3-4 times, then centrifuge at 600 *g* for 5 minutes at 4^oC,dicard supernatant.
   Note: The resulting pellet is the nuclei, cellular debris and so on.

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com

4. Add 0.4ml ice cold Hypotonic Buffer (before use ,add 1ul DTT and 5ul Phosphatase inhibitor ,10ul PMSF into1ml Hypotonic Buffer), vortex for 10 seconds, then centrifuge at 3,000 *g* for 5 minutes at 4°C, dicard supernatant for washing away residual cytosolic protein and cellular debris.
5. Add 0.2ml ice cold Lysis Buffer (before use ,add 1ul DTT and 5ul Phosphatase inhibitor ,10ul PMSF into1ml Lysis Buffer) to precipitates, place them on icebag for 20 minutes, occasionally vortex for 3-4 times, then centrifuge at 18,000 *g* for 5 minutes at 4°C, keep supernatant as Nuclear Protein fraction.

Notes:

1. All of reagents and instruments must be pre-cold, thus the extracted protein can remain activity and intact.
2. For common experiments, do not need dialysis, but for accurate experiments, you must dialysis to remove salt.
3. For nuclear protein quantitation, can use Non-Interfering Protein Concentration Determination Kit (SK3071) or Better BCA Protein Assay Kit (SK3051).
4. If low cytosolic protein concentration in nuclear protein fraction ,maybe carry out step 4 once more for diminishing cross-contamination of cytoplasmic proteins in the Nuclear Protein fraction.
5. If Low protein concentration in nuclear Protein fractions, please add 5ul Phosphatase inhibitor, 1ul DTT,10ul PMSF into 1ml Lysis Buffer before use.

20 Konrad Cres, Markham Ontario L3R 8T4 Canada
Tel: (905) 474 4493, (800) 313 7224 Fax: (905) 474 5794
Email: order@biobasic.com Web: www.biobasic.com