

ClearBand Cytoplasmic & Nuclear Protein Fractionation Kit

Troubleshooting Guide

100 extractions from cultured cells or 20 mg tissue samples

Cat No: CNF100

Use this guide for common fractionation problems when preparing cytoplasmic and nuclear extracts from fresh mammalian cultured cells or tissue. The protocol is designed for up to 1×10^6 cells or 20 mg fresh tissue per extraction and requires cold handling, staged CE/NE buffers, and repeated nuclear-pellet washing.

Before You Start - Quick Checks

1. Use fresh cells or tissue and keep samples, buffers, and extracts cold throughout the procedure.
2. Perform centrifugation steps at 4°C and use pre-chilled collection tubes where specified.
3. Add protease inhibitors to CE I and NE I immediately before use; they are not required in CE II or NE II.
4. Scale all reagent volumes proportionally if the sample amount is increased beyond the standard input.

Problem	Most likely cause	Recommended action
High nuclear contamination in the cytoplasmic fraction	The pellet may have been disrupted during supernatant transfer or the fractionation steps may not have been followed cleanly.	Transfer the cytoplasmic supernatant immediately and carefully without disturbing the insoluble nuclear pellet.
High cytoplasmic contamination in the nuclear fraction	CE II wash steps may have been incomplete or residual supernatant may have remained on the nuclear pellet.	Repeat the CE II wash/centrifugation sequence twice as specified and remove the supernatant thoroughly before NE extraction.
Low cytoplasmic protein yield	The sample amount, CE I volume, resuspension, or initial vortexing may be inadequate.	Use about 1×10^6 cells or 20 mg tissue, approximately 5 volumes of ice-cold CE I, and obtain a homogeneous suspension before centrifugation.
Low nuclear protein yield	The nuclear pellet may not have been fully extracted with NE I/NE II or the homogenization/vortex sequence may be incomplete.	Use 50 μ L NE I plus 20 μ L NE II for the standard input and complete the bead homogenization and repeated vortexing schedule.
Protein degradation is observed	Protease inhibitors may have been omitted or samples may have warmed during processing.	Add protease inhibitors to CE I and NE I immediately before use, keep samples on ice, and centrifuge at 4°C.
Pellet is too wet before the next extraction step	Residual wash or supernatant was not removed completely.	Carefully remove the supernatant after each centrifugation and leave the pellet as dry as practical before adding the next buffer.
Fractionation quality decreases with larger samples	The sample amount was increased without scaling reagent volumes.	Scale all reagent volumes proportionally when using more than the standard cell or tissue input.

Table continues on next page.

ClearBand Cytoplasmic & Nuclear Protein Fractionation Kit Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Tissue fractionation is inconsistent	Tissue may not have been cut and homogenized thoroughly in CE I.	Use fresh tissue, cut it into small pieces, and homogenize in about 5 volumes of ice-cold CE I before proceeding.
Nuclear pellet remains visibly heterogeneous	The NE extraction suspension may not be fully homogenized.	Extend the vortexing step if necessary and continue 15-second vortexing every 10 minutes for a total of 30 minutes on ice.
Downstream nuclear assay performs poorly	The nuclear extract may contain excess salts for the downstream application.	If large nuclear-extract volumes are used or downstream problems occur, dialyze the nuclear extract to remove excess salts.
Extract loses activity during handling	Samples may have remained warm or unprotected.	Keep extracts on ice during immediate use and store aliquots at -80°C for future use.
Cross-contamination exceeds expectations	Critical wash, transfer, or pellet-handling steps may have been shortened.	Repeat the workflow with careful phase transfer and the full two CE II washes; the kit is designed to generally yield less than 10% cross-contamination.
Protease inhibitor was added to every buffer	The datasheet specifies inhibitor addition to CE I and NE I, not CE II or NE II.	Use concentrated inhibitor in CE I and NE I immediately before use and leave CE II/NE II unchanged unless the protocol is separately validated.
Fresh sample is unavailable	The kit is intended for fresh cells or tissue.	Use fresh starting material when following the supplied protocol rather than assuming archived material will behave identically.

Still having trouble? Recheck fresh starting material, sample amount, proportional buffer volumes, 4°C centrifugation, protease inhibitors in CE I/NE I, CE II washes, and cold handling before changing the downstream assay.