
ReadyPrep™ Protein Extraction Kit (Cytoplasmic/Nuclear)

Instruction Manual

Catalog #163-2089

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Section 1

Introduction

The ReadyPrep protein extraction kit (cytoplasmic/nuclear) is designed to quickly prepare highly enriched fractions of cytoplasmic and nuclear proteins from eukaryotic samples, such as cultured cells and tissues. This kit is part of a group of related kits from Bio-Rad developed to reduce sample complexity in order to improve the chances of identifying low abundant proteins and to simplify proteomic studies. Although this kit is directed towards the preparation of nuclear and cytoplasmic protein samples for 2-D gel electrophoresis, the fractions are suitable for many applications such as SDS-PAGE and western blotting.

The ReadyPrep protein extraction kit (cytoplasmic/nuclear) utilizes a specially formulated buffer and differential centrifugation to isolate intact nuclei (Dignam et al. 1983, Zerivitz and Akusjarvi 1989). The kit also contains a strongly chaotropic extraction buffer to efficiently solubilize nuclear proteins for immediate use in 2-D gel electrophoretic applications. Analysis of the cytoplasmic fraction requires that the protein first be processed through the ReadyPrep 2-D cleanup kit, which is provided. This kit removes components from the extraction buffer that would interfere with the isoelectric focusing step.

Section 2

Kit Specifications

Each ReadyPrep protein extraction kit (cytoplasmic/nuclear) provides sufficient reagents to perform up to 50 extractions of 50 mg of cells or tissue. The procedure can easily be scaled up to accommodate larger amounts of cells or tissue.

Items Supplied With Kit

One bottle containing 50 ml of cytoplasmic protein extraction buffer (CPEB)

One bottle containing 25 g of protein solubilization buffer (PSB)

One bottle containing 30 ml of PSB diluent

One ReadyPrep 2-D cleanup kit

Items Required But Not Provided

- 1.5 ml microcentrifuge tubes
- Microcentrifuge capable of spinning at 12-16,000 x g at 4°C.
- Vortex mixer
- Homogenizer/tissue grinder.
 - 20 gauge syringe needle (for cultured cells)
 - Wheaton Dounce tissue homogenizer (for example, VWR catalog #62400-595)

- Wheaton Potter-Elvehjem tissue grinder (for example, VWR catalog #62400-700)
- Kontes tight-fitting microcentrifuge-pestle combination (for example, VWR catalog #KT749520-0000)
- 1X Phosphate buffered saline (for cultured cells)
- Protease inhibitor(s)
- Reducing agent (for example, DTT, TBP, or TCEP)
- Carrier ampholytes (for example, Bio-Lyte[®] 3/10 ampholyte)
- *RC DC*[™] Protein Assay (Bio-Rad catalog #500-0121 or #500-0122)

Section 3

Storage Conditions

The unopened kit can be stored at room temperature. CPEB and PSB diluent should be stored at 2–8°C after opening. Use aseptic technique when handling CPEB and PSB diluent to prevent contamination. For storage of the ReadyPrep 2-D cleanup kit, follow the instructions provided in the kit.

Section 4

Instructions for Use

Two protocols have been provided. They differ based on the type of starting sample material – one for cultured cells, one for tissue samples. Use the protocol that best fits the experimental sample. Please read the entire protocol before using the kit.

4.1 Protocol A

4.1.1 Extraction of Cytoplasmic/Nuclear Proteins From Cultured Cells

Note: Chill CPEB on ice for at least 15 min before beginning.

1. Transfer cell culture suspension or adherent cells (1–5 ml) into a 1.5 ml microcentrifuge tube and centrifuge to obtain approximately 0.05 ml of packed cells. Remove and discard the supernatant.
2. If the cells have not previously been washed, add 1 ml of ice-cold 1X phosphate-buffered saline solution to the cell pellet. Pipet up and down several times or vortex briefly to resuspend the cells, and pellet the cells again by centrifugation. Remove and discard the wash solution.

3. For each 0.05 ml of packed cells, add 0.5 ml of ice-cold CPEB. Vortex to suspend the cell pellet and incubate the cells on ice for 30 min.

Notes: Protease inhibitors may be added to CPEB immediately prior to use to prevent proteolysis during the extraction process.

Insufficient volume of CPEB may result in poor cell lysis, low cytoplasmic protein yield, and contamination of the nuclear pellet with cytoplasmic proteins.

4. Gently pass the cell suspension through a narrow-opening syringe needle (20 gauge) to lyse the cells without damaging the nuclei. Slowly force the cells through the needle for 10–20 strokes to ensure complete cell lysis.

Note: Care should be exercised when breaking open the cells; excessive handling and severe force may damage the nuclei, releasing nuclear proteins into the cytoplasmic fraction. Some optimization of the handling procedure may be required.

5. Centrifuge the cell lysate at 1000 x g for 10 min at 4°C. Upon completion of the centrifugation, use a pipet to immediately transfer the supernatant containing the cytoplasmic proteins to a new tube (on ice) labeled Cytoplasmic Protein Fraction. Centrifuge the original tube for 5–10 sec at 1000 x g, remove any remaining liquid, and pool it with the previous supernatant.

6. The pellet in the tube from step 5 contains nuclei. Wash the nuclear pellet one time with 0.25 ml of CPEB (see note below). Vortex briefly or gently pipet up and down to resuspend the nuclei and incubate on ice for 10 min. Centrifuge as in step 5 to concentrate the nuclei and either pool the wash supernatant with the previous Cytoplasmic Protein Fraction or discard.

Notes: Washing of the nuclei is critical to minimize contamination of the nuclei with cytoplasmic proteins. If desired, step 4 can be repeated after resuspending the nuclear pellet to ensure that all cells have been broken open and that cytoplasmic proteins are not introduced into the nuclear fraction via cellular contamination of the nuclear pellet. Insufficient washing of the nuclei can lead to failure in the enrichment of nuclear proteins and result in 2-D gels where the cytoplasmic and nuclear fractions produce very similar protein spot patterns.

If no further downstream applications are to be immediately performed, aliquot and quick-freeze the cytoplasmic protein fraction on dryice and then store at -70°C. Also, to avoid repeated freeze-thawing of the sample, it is often convenient at this stage to set aside a 5–10 µl aliquot of the cytoplasmic protein fraction before freezing for protein quantitation. This amount is sufficient for the Bio-Rad RC DC protein assay.

7. Prepare a fresh volume of complete PSB sufficient to suspend the nuclear pellet formed in step 6 (a total of 0.75 ml is needed per 0.05 ml of original packed cells). See Section 5.2 for instructions on preparing the complete PSB.

8. Suspend the nuclei in 0.5 ml of complete PSB. Vortex the tube 4–5 times, 60 sec each, to solubilize the nuclear proteins. Genomic DNA should be evident in the sample tube at this stage. Centrifuge at maximum speed (12–16,000 x g) for 15–20 min at room temperature to pellet the genomic DNA and other debris and transfer the clarified supernatant into a new microcentrifuge tube labeled Nuclear Protein Fraction.
9. Reextract the residual pellet from step 8 using approximately one-fourth to one-half the volume of complete PSB used previously (0.13–0.25 ml). Centrifuge at maximum speed for 15–20 min at room temperature, collect the clarified supernatant, and pool it with the previous nuclear protein fraction.

4.1.2 Processing Cytoplasmic and Nuclear Protein Fractions for IEF/2-D Analysis

10. Determine the protein concentration of the the cytoplasmic and nuclear protein fractions. The *RC DC* protein assay is recommended for this measurement. This assay allows accurate protein quantitation in the presence of detergents, reducing agents, and other substances that typically interfere with other protein assays.

11. Make an appropriate dilution of the nuclear protein fraction into complete PSB or other strongly chaotropic 2-D rehydration/sample buffer before performing IEF/2-D gel electrophoresis. Refer to Section 5.1 for guidelines on selecting the appropriate volume of complete PSB or 2-D rehydration/sample buffer and Section 5.2 for directions on preparing PSB and other strongly chaotropic 2-D rehydration/sample buffers.
12. Process an appropriate amount of the cytoplasmic protein fraction through the ReadyPrep 2-D cleanup kit. To determine an appropriate amount, refer to the guidelines in Section 5.1. Follow the ReadyPrep 2-D cleanup kit protocol provided. At the end of the 2-D cleanup procedure, resuspend the cytoplasmic protein pellet in the appropriate amount of complete PSB or other 2-D rehydration/sample buffer and perform IEF/2-D analysis.

4.2 Protocol B

4.2.1 Extraction of Cytoplasmic/Nuclear Proteins From Tissue Samples

Note: For best results, use a Wheaton Dounce tissue homogenizer or Potter-Elvehjem tissue grinder. See Section 2 for catalog information.

1. Chill the Dounce in ice before beginning. Add ~50 mg of tissue into the chilled Dounce homogenizer. Add 0.75 ml of CPEB to the tissue (~15 ml/gm of tissue).

Notes: If using a tight-fitting microcentrifuge-pestle combination, use 0.5 ml of CPEB per 50 mg of tissue and completely homogenize the tissue (5–15 gentle strokes). Proceed to step 3.

Protease inhibitors may be added to CPEB immediately prior to use to prevent proteolysis during extraction.

Insufficient volume of CPEB may result in poor cell lysis, low cytoplasmic protein yield, and contamination of the nuclear pellet with cytoplasmic proteins.

2. If using a Dounce homogenizer, break up the tissue with 5–15 strokes using the loose-fitting pestle. Then, complete the release of the nuclei from the cells with 8–10 strokes using the tight-fitting pestle. If using a Potter-Elvehjem type tissue grinder, homogenize the tissue completely with 15–25 strokes of the tight-fitting pestle. In either case, do not twist the pestle while raising and lowering, as this may rupture the nuclei

and contaminate the cytoplasmic protein fraction with nuclear proteins.

Note: Certain tissues may require additional homogenization to achieve complete cell lysis. Some optimization may be required.

3. Incubate the Dounce or tissue grinder containing the homogenate on ice for 1–2 min to allow any large tissue fragments to sediment to the bottom without pelleting the nuclei. Using a pipet, carefully transfer the supernatant to a clean tube, leaving behind any cellular debris at the bottom of the vessel.
4. Proceed beginning at Step 5 in Section 4.1.1 to complete the nuclear isolation and solubilization procedure.

Section 5

Appendix

5.1. Rehydration/Sample Buffer Volume

In the final step for this kit, all samples are resuspended in a 2-D rehydration/sample buffer (Section 5.2). To best determine the volume of 2-D rehydration/sample buffer to use, consider the questions listed below. To assist with these calculations, the table that follows indicates appropriate volumes of 2-D rehydration/sample buffer needed to rehydrate IPG strips of specific lengths and the approximate amounts of protein required for detection using silver stain or Coomassie Blue G-250 stain. An example illustrates how to calculate the volume of 2-D rehydration/sample buffer required.

1. What is the quantity of protein precipitated in the tube?
2. For 2-D electrophoresis experiments using IPG strips, what length strip will be used?
3. What is the pH range of the IPG strip to be used?
4. How complex is the protein sample?
5. What staining or detection method will be used?
(for example, Bio-Safe™ Coomassie stain, silver stain, etc.)

IPG strip length	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume per strip	125 μ l	185 μ l	300 μ l	315 μ l	410 μ l
Protein load– Silver stain	5–20 μ g	20–50 μ g	50–80 μ g	50–80 μ g	80–150 μ g
Protein load– Coomassie G-250	50–100 μ g	100–200 μ g	200–400 μ g	200–400 μ g	400–800 μ g

Sample calculation: If you precipitate 100 μ g of protein and are going to run 7 cm pH 3–10NL IPG strips (125 μ l per strip) and silver stain the 2-D gels, then you may want to solubilize the protein pellet in ~900 μ l of rehydration/sample buffer, which is enough to rehydrate about seven 7 cm IPG strips (~14 μ g/strip). However, if you are planning to use a 24 cm pH 3–10NL IPG strip, then you may want to solubilize the protein pellet in 410 μ l of rehydration/sample buffer, which is enough to rehydrate one 24 cm IPG strip (100 μ g/strip). In this simple example, sample complexity and IPG strip pH range were not addressed. As a general rule, increased protein loads may be required for micro-range IPG strips and for samples of higher protein complexity.

5.2 Preparation of 2-D Rehydration/Sample Buffers

The PSB and PSB diluent are provided with this kit to solubilize the nuclear proteins in the nuclear pellet. PSB is a proprietary, strongly chaotropic 2-D rehydration/sample buffer that will solubilize both hydrophilic as well as hydrophobic proteins. Other strongly chaotropic 2-D rehydration/sample buffers (Section 5.2.2) may be substituted for PSB or used for diluting an appropriate amount of sample for IEF/2-D analysis.

5.2.1. Complete Protein Solubilization Buffer (PSB)

To make 2 ml of complete 2-D rehydration/sample buffer, add 1.1 ml of PSB diluent to each 1 g of PSB powder.

Note: Before weighing out the PSB powder, shake the bottle vigorously 10-15 seconds to break up any clumps and to ensure a uniform blend of the different components.

Mix the solution until the powder is completely dissolved (the tube can be warmed to speed dissolution of the solids, but do not allow the temperature to exceed 30°C). Add DTT, Bio-Lyte ampholyte, and Bromophenol Blue according to the table below to complete the preparation of the buffer.

Component	Final Concentration	Amount to Make 2 ml
DTT* (FW 154.3)	50 mM	15.4 mg
100X Bio-Lyte 3/10 ampholyte**	0.2% (w/v)	20 μ l
Bromophenol Blue	0.002% (w/v)	4 μ l of a 1% (w/v) solution

*If TBP or TCEP is substituted for DTT as the reducing agent, use at a concentration of 2 mM.

**Use an ampholyte buffer that corresponds to the pH range of the IEF separation to be performed. For example, ReadyStrip™ micro-range buffers with ReadyStrip micro-range IPG strips and ReadyStrip 7-10 buffer with ReadyStrip pH 7-10 IPG strips. Bio-Lyte 3/10 ampholyte can be used with all other ReadyStrip IPG strip pH ranges.

5.2.2. Strongly Chaotropic 2-D Rehydration/Sample Buffer

(7 M urea, 2 M thiourea, 4% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte, 0.002% Bromophenol Blue).

Component	Final Concentration	Amount to make 2 ml
Urea (FW 60.06)	7 M	0.84 g
Thiourea (FW 76.12)	2 M	0.304 g
CHAPS [*]	4% (w/v)	0.08 g
DTT (FW 154.3)	50 mM	15.4 mg
100X Bio-Lyte 3/10 ampholyte ^{**}	0.2% (w/v)	20 μ l
Bromophenol Blue	0.002% (w/v)	4 μ l of a 1% (w/v) solution
Proteomic grade water		1.1 ml

^{*}Other neutral or zwitterionic detergents can also be used at concentrations of 1% to 2% (w/v) to improve solubilization of membrane and hydrophobic proteins. Examples are n-octyl- β -D-glucopyranoside, SB3-10 (N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate) and ASB14 (tetradecanoylamidopropyl-dimethylammonio-propane-sulfonate).

^{**}Use an ampholyte buffer that corresponds to the pH range of the IEF separation to be performed. For example, ReadyStrip[™] micro-range buffers with ReadyStrip micro-range IPG strips and ReadyStrip 7-10 buffer with ReadyStrip pH 7-10 IPG strips. Bio-Lyte 3/10 ampholyte can be used with all other ReadyStrip IPG strip pH ranges.

Section 6

References

1. Dignam JD et al., Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acid Res* 11, 1475–1489 (1983).
2. Zerivitz K and Akusjarvi G, An improved nuclear extract preparation method. *Gene Anal Tech.* 6, 101–109 (1989).

Section 7

Product Information

Catalog #	Description
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Sample Preparation Kits

163-2130	ReadyPrep 2-D Cleanup Kit, 50 preps
163-2089	ReadyPrep Protein Extraction Kit (Cytoplasmic/Nuclear), 50 preps
163-2088	ReadyPrep Protein Extraction Kit (Membrane I), 50 preps
163-2087	ReadyPrep Protein Extraction Kit (Signal), 50 preps
163-2090	ReadyPrep Reduction Alkylation Kit
163-2100	ReadyPrep Sequential Extraction Kit, 5–15 preps

Protein Quantitation Kits (also see bulletin 2610)

500-0121	<i>RC DC</i> Protein Assay Kit I, 500 standard assays, bovine γ -globulin standard
500-0122	<i>RC DC</i> Protein Assay Kit II, 500 standard assays, bovine serum albumin standard

Buffer Components

161-0611	Dithiothreitol (DTT), 5 g
163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml
161-0460	CHAPS, 1 g
161-0731	Urea, 1 kg
161-0716	Tris, 500 g
161-0302	Sodium Dodecyl Sulfate (SDS), 1 kg
163-2094	100X Bio-Lyte 3/10 Ampholyte, 1 ml
163-2091	ReadyPrep Proteomic Grade Water

Rehydration/Sample Buffers

163-2106	ReadyPrep 2-D Starter Kit Rehydration/Sample Buffer, 10 ml, containing 8 M urea, 2% CHAPS, 50 mM DTT, 0.2% Bio-Lyte 3/10 ampholyte, Bromophenol Blue
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