Nuclear Extract Kit

(version D5)

Catalog Nos. 40010 & 40410

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Revision History

Revision	Date	Description of Change
D5	May 2022	
IMPORTANT: Phosphatase Inhibitors (AM Part 102146) has been replaced with 100X Phosphatase Inhibitors (AM Part 105840). Consult the Quick Charts for the appropriate protocol (nuclear, cytoplasmic or whole-cell extract from cells or from tissue) to determine the updated volumes that will be required for your extraction(s).		
D4	Dec 2018	Clarified recommendations for measuring protein concentration of the cell extract

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Introduction

The Nuclear Extract Kit has been developed for the preparation of nuclear, whole-cell and cytoplasmic extracts from cells or tissue. The kit procedure is a simple, fast and effective way to obtain non-denatured, active proteins contained in cytoplasmic and nuclear compartments of the cell. The proteins collected by this high-salt extraction method can be used for a variety of standard protocols including electrophoretic mobility shift assay (EMSA), DNA footprinting, Western blotting and preparative purification of nuclear proteins for use in downstream applications such as enzyme activity assays or binding studies. The Nuclear Extract Kit is the recommended method for preparation of cell extracts for use in Active Motif's TransAM™ assays to monitor transcription factor activation.

Each kit provides reagents for 100 or 400 extractions from 8.8 x 10^6 cells, which corresponds to HeLa cells grown to confluence in a 100 mm tissue culture dish.

The Nuclear Extract Kit is intended for use for preparation of nuclear, cytoplasmic and whole-cell fractions from various cell and tissue types. Extractions can be performed with fresh and frozen cell and tissue samples.

For preparation of nuclear extracts, the cells are first collected in ice-cold PBS in the presence of phosphatase inhibitors to limit further protein modifications (expression, proteolysis, dephosphorylation, etc.). Then, the cells are resuspended in Hypotonic Buffer to swell the cell membrane and make it fragile. Addition of the Detergent causes leakage of the cytoplasmic proteins into the supernatant. After collection of the cytoplasmic fraction, the nuclei are lysed and the nuclear proteins are solubilized in detergent-free Lysis Buffer in the presence of the Protease Inhibitor Cocktail.

To prepare whole-cell extracts, cells are collected in the PBS/Phosphatase Inhibitors solution and lysed in the Lysis Buffer. Solubilized proteins are separated from the cell debris by centrifugation.

To prepare extracts from fresh or frozen tissue, samples are diced and disrupted using a Dounce homogenizer to form a single-cell slurry. Cells are then lysed in the lysis buffer and solubilized proteins are separated from the cell debris by centrifugation.

The protein concentration of the cell extract is then measured by Active Motif's ProStain™ Protein Quantification Kit (Catalog No. 15001) or a Bradford-based assay. We do not recommend the BCA assay as the appropriate dilution required will produce such a dilute extract, the protein concentration will not be within the range of the standard curve produced.

Kit Performance and Benefits

The Nuclear Extract Kit is for research use only. Not for use in diagnostic procedures.

Assay time: 2 hours

Yield of protein: Cytoplasmic extract: ~0.5-1 mg at ~1-2 mg/ml from 8.8 x 10^6 cells
Nuclear extract: ~0.15-0.25 mg at ~3-5 mg/ml from 8.8 x 10^6 cells
Whole-cell extract: ~1.2-2.4 mg at 4-8 mg/ml from 8.8 x 10^6 cells

(Note: Example is from HeLa cells grown to confluency)

Assay compatibility: TransAM™, EMSA, DNA footprinting, Western blotting, preparative nuclear protein purification, protein assays.
Active transcription factors extracted include NFκB, AP-1, CREB, p53, HIF-1, STAT, Sp1, NFAT, MyoD, NF-YA, C/EBP and PPARγ.
Successful extraction has been performed with HeLa, WI-38, COS-7, PC-12, Jurkat and RINm5F cells, and many more. Extraction can be performed with fresh or frozen cell and tissue samples.

Time Course Studies: For time course studies where the effectors are added at different timepoints, we suggest that cells be lysed and processed at the same time to maintain consistency among samples.

Nuclear Extract Kit

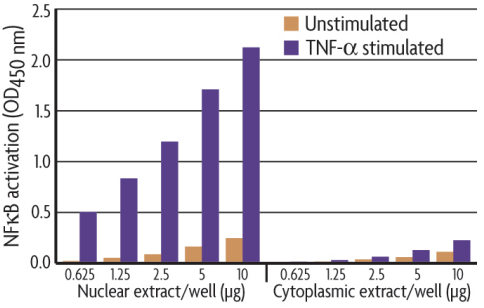


Figure 1: Specific extraction of nuclear and cytoplasmic extracts.

NFκB activation was assayed with the TransAM® NFκB p50 Kit (Catalog Nos 41096 and 41596) using increasing amounts of nuclear and cytoplasmic extracts isolated from unstimulated and stimulated HeLa cells using the Nuclear Extract Kit.

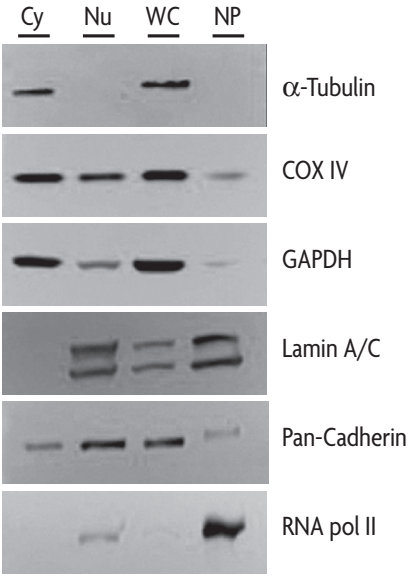


Figure 2: Western blots of specific proteins from cellular fractions.

Active Motif's Nuclear Extract Kit was used to generate nuclear, cytoplasmic and whole-cell extracts from HeLa cells to analyze the efficiency of cellular fractionation. 20 µg of cytoplasmic (Cy), nuclear (Nu), and whole-cell (WC) extracts, along with the nuclear pellet (NP) collected from nuclear extraction, were run on 4-20% SDS-PAGE gels and analyzed by Western blot using 1:1000 dilutions of primary antibodies to various specific proteins. Primary antibodies: Active Motif alpha Tubulin mAb, Catalog No. 39528; COX IV mAb; GAPDH mAb; Active Motif Lamin A/C mAb, Catalog No. 39288; Pan-Cadherin mAb; Active Motif RNA pol II mAb, Catalog No. 39097. Note that RNA pol II is found primarily in the nuclear pellet due to its association with the nucleoskeleton and chromatin. The optional addition of Detergent to the nuclear pellet can sometimes aid in solubilizing nucleoskeleton- or chromatin-associated proteins such as RNA pol II.

Kit Components and Storage

Kit components can be stored at –20ºC prior to first use. Then, we recommend storing each component at the temperatures recommended in the table below:

Reagents	Quantity 100 rxns / 400 rxns	Storage/Stability
Lysis Buffer AM1	10 ml / 50 ml	4°C for 6 months
1 M Dithiothreitol (DTT)	100 µl / 500 µl	-20°C for 1 year
Protease Inhibitor Cocktail	100 µl / 500 µl	-20°C for 1 year
10X PBS	100 ml / 4 x 100 ml	4°C for 6 months
100X Phosphatase Inhibitors	8 ml / 4 x 8 ml	-20°C for 6 months
10X Hypotonic Buffer	50 ml / 4 x 50 ml	4°C for 6 months
Detergent	3 ml / 4 x 3 ml	4°C for 1 year

Additional Materials Required

5 and 10 ml pipettes

Pipettors

Cell scraper

15 ml conical tubes

Microcentrifuge tubes

Centrifuge (with swinging buckets adapted to 15 ml conical tubes) and microcentrifuge pre-cooled at 4ºC

Rocking platform

Distilled water

Optional Additional Materials

Dounce homogenizer, 2 ml capacity, with a large (looser, A) pestle clearance of 0.12 mm for the initial sample reduction, and a small (tighter, B) pestle clearance of 0.06 mm for the final homogenate

Note: Adherent and suspension cell preparations require the small-clearance (B) pestle whereas fresh and frozen tissue preparations require both large (A)- and small (B)-clearance pestles.

(Suggested Dounce homogenizer: Active Motif, Catalog Nos. 40401 and 40415)

Reagents from the Nuclear Extract Kit

IMPORANT: As of May, 2022, there is a new formulation of Phosphatase Inhibitors. The originial Phosphatase Inhibitors (AM Part 102146), has been replaced by 100x Phosphatase Inhibitors (AM Part 105840). Consult the appropriate Quick Chart (nuclear, cytoplasmic or whole-cell extract from cells or from tissue) to determine the updated volumes for each solution.

Preparation of PBS/Phosphatase Inhibitors

To make nuclear extract from a 100 mm plate of cells, prepare 8 ml of PBS/Phosphatase Inhibitors solution as follows: mix 0.8 ml 10X PBS in 7.12 ml distilled water, then add 80 µl 100X Phosphatase Inhibitors. Since the Phosphatase Inhibitors lose their activity 24 hours after dilution, the solution should be used within this time frame. Any remaining solution should be discarded if not used on the same day.

Preparation of 1X Hypotonic Buffer

Note that the Hypotonic Buffer already contains phosphatase (but not protease) inhibitors. Consult the protocol you intend to use (nuclear, cytoplasmic or whole-cell extract from cells or from tissue) to determine the amount of 1X Hypotonic Buffer that will be required for your extraction(s). For example, to make nuclear extract from a 100 mm plate of cells, prepare 500 µl of 1X Hypotonic Buffer as follows: mix 50 µl 10X Hypotonic Buffer and 450 µl distilled water. Any remaining 1X Hypotonic Buffer can be stored at 4ºC for 1 week.

Preparation of 10 mM DTT

The Nuclear Extract Kit is supplied with 1 M DTT. To make Complete Lysis Buffer, you will need to first dilute the 1 M DTT to create 10 mM DTT. However, 1 M DTT is used to perform nuclear and cytoplasmic extractions from tissue (see Protocol II: Fresh or Frozen Tissue on page 10). To make 10 mM DTT, make a 1:100 dilution of the supplied 1 M DTT in distilled water. For example, add 1 µl of 1 M DTT to 99 µl distilled water. DTT is highly labile, so 10 mM DTT must be prepared fresh each time. Avoid multiple freeze/thaw cycles.

Preparation of Complete Lysis Buffer

Note that the Complete Lysis Buffer already contains phosphatase (but not protease) inhibitors. The presence of phosphatase inhibitors gives a yellow coloration to Lysis Buffer AM1. Consult the appropriate protocol (nuclear, cytoplasmic or whole-cell extract from cells or from tissue) to determine the amount of Complete Lysis Buffer that will be required for your extraction(s). For example, to make nuclear extract from a 100 mm plate of cells, prepare 50 µl Complete Lysis Buffer as follows: add 5 µl 10 mM DTT to 44.5 µl of Lysis Buffer AM1, then add 0.5 µl Protease Inhibitor Cocktail. Since some of the protease inhibitors lose their activity promptly after dilution. Therefore, use the Complete Lysis Buffer immediately for cell lysis. Any remaining amount should be discarded if not used on the same day.

Protocol I: Adherent or Suspension Cells

A. Preparation of Nuclear Extract from Cells

The following protocol is based on samples of approximately 8.8 x 10^6 cells, which correspond to HeLa cells grown to confluence in a 100 mm tissue culture plate. Each sample is one reaction. Prepare PBS/Phosphatase Inhibitors, Hypotonic Buffer and Complete Lysis Buffer as described above on page 5 in the section Buffer Preparation. Adjust the volumes according to the buffer preparation chart if using plates of different sizes. Place buffers and any tubes needed on ice before beginning assay.

Quick Chart for Preparing Buffers for Nuclear Extraction from Cells

Reagents to Prepare	Components	60 mm plate or 3.2 x 10^6 cells	100 mm plate or 8.8 x 10^6 cells	150 mm plate or 2 x 10^7 cells
PBS/Phosphatase Inhibitors	10X PBS	0.4 ml	0.8 ml	1.6 ml
	Distilled water	3.56 ml	7.12 ml	14.24 ml
	100X Phosphatase Inhibitors	40 µl	80 µl	160 µl
	TOTAL REQUIRED	**4.0 ml**	**8.0 ml**	**16.0 ml**
1X Hypotonic Buffer	10X Hypotonic Buffer	25 µl	50.0 µl	100 µl
	Distilled water	225 µl	450.0 µl	0.9 ml
	TOTAL REQUIRED	**250 µl**	**500 µl**	**1 ml**
Complete Lysis Buffer	10 mM DTT	2.5 µl	5 µl	10.0 µl
	Lysis Buffer AM1	22.25 µl	44.5 µl	89 µl
	Protease Inhibitor Cocktail	0.25 µl	0.5 µl	1.0 µl
	TOTAL REQUIRED	**25 µl**	**50 µl**	**100 µl**
*(Optional) Detergent	**TOTAL REQUIRED**	**1.25 µl**	**2.5 µl**	**5 µl**

*The addition of Detergent to the nuclear pellet may help with solubility of proteins, specifically those tightly associated with membranes or chromatin.

Step 1: Cell Collection

1. Aspirate media out of dish. Wash with 5 ml ice-cold PBS/Phosphatase Inhibitors. Aspirate solution out and add 3 ml ice-cold PBS/Phosphatase Inhibitors. If working with suspension cells, pellet cells and wash with 3 ml ice-cold PBS/Phosphatase Inhibitors and proceed to step 3.
2. Remove cells from dish by gently scraping adherent cells with cell lifter or, alternatively, pellet suspension cells by centrifugation. Transfer cells to a pre-chilled 15 ml conical tube.

   Note: Use of trypsin to detach cells can activate some signal transduction pathways.

3. Centrifuge cell suspension for 5 minutes at 200 x *g* in a centrifuge pre-cooled at 4ºC.
4. Discard supernatant. Keep cell pellet on ice.

Step 2: Cytoplasmic Fraction Collection

Note: Make sure to verify cell lysis efficiency and release of the nuclei by comparing the appearance of cells before and after cell lysis (steps 1 and 2) using phase-contrast microscopy. Intact cells should appear as a dark central nucleus surrounded by a halo of less dense cytoplasm.

1. Gently resuspend cells in 500 µl 1X Hypotonic Buffer by pipetting up and down several times. Transfer to a pre-chilled microcentrifuge tube. Allow cells to swell by incubating for 15 minutes on ice.
2. Add 25 µl Detergent (for tissue, add 5 µl Detergent per every 100 µl Hypotonic Buffer) and vortex 10 seconds at the highest setting.
3. Check a small sample under the microscope to verify that cells have been efficiently lysed and that nuclei have been released. If the cells are not adequately lysed at this step, use an ice-cold Dounce homogenizer with a small-clearance (B) pestle to lyse the cells (refer to the Troubleshooting Guide in Section A of the Appendix, page 14, for further information).

   Note: Do not proceed with the centrifugation step until the cells are sufficiently lysed.

4. Centrifuge suspension for 30 seconds at 14,000 x *g* in a microcentrifuge pre-cooled at 4ºC.
5. Transfer supernatant (cytoplasmic fraction) into a pre-chilled microcentrifuge tube. Store the supernatant at –80ºC until ready to use. Use the pellet for nuclear fraction collection. OPTIONAL: Save a fraction of the supernatant (cytoplasmic fraction) for determination of fractionation efficiency by Western blot.

Step 3: Nuclear Fraction Collection

1. Resuspend nuclear pellet in 50 µl Complete Lysis Buffer by pipetting up and down. OPTIONAL: Add 2.5 µl Detergent to help solubilize membrane-associated nuclear proteins. A very viscous pellet may form and not completely resuspend. Alternatively, a Dounce homogenizer can be used (refer to the Troubleshooting Guide in Section A of the Appendix, page 14, for further information). Vortex 10 seconds at the highest setting.
2. Incubate suspension for 30 minutes on ice on a rocking platform set at 150 rpm.
3. Vortex 30 seconds at the highest setting. Centrifuge for 10 minutes at 14,000 x *g* in a microcentrifuge pre-cooled at 4ºC. Transfer supernatant (nuclear fraction) into a pre-chilled microcentrifuge tube. OPTIONAL: Save a fraction of the supernatant (nuclear fraction) for determination of fractionation efficiency by Western blot.
4. Aliquot and store at –80ºC. Avoid freeze/thaw cycles.

   Note: The presence of certain detergents or PMSF may interfere with the Bradford assay, thus perform a 1:50 or 1:250 dilution of your samples. Use the Complete Lysis Buffer as the blank with a similar dilution as the samples. Read the absorbance at 595 nm with a plate reader spectrophotometer (refer to the Appendix, Section A on page 13 for an example dilution series page 13). As an alternative, try Active Motif's ProStain™ Protein Quantification Kit (Catalog No 15001), which offers greater sensitivity and resistance to many contaminating agents.

B. Preparation of Whole-Cell Extract from Cells

The following protocol is based on samples of approximately 8.8 x 10^6 cells, which correspond to HeLa cells grown to confluence in a 100 mm tissue culture plate. Each sample is one reaction. Prepare PBS/Phosphatase Inhibitors and Complete Lysis Buffer as described above on page 5 in the section Buffer Preparation. Adjust the volumes according to the buffer preparation chart if using plates of different sizes. Place buffers and any tubes needed on ice before beginning assay.

Quick Chart for Preparing Buffers for Whole-Cell Extraction from Cells

Reagents to Prepare	Components	60 mm plate or 3.2 x 10^6 cells	100 mm plate or 8.8 x 10^6 cells	150 mm plate or 2 x 10^7 cells
PBS/Phosphatase Inhibitors	10X PBS	0.4 ml	0.8 ml	1.6 ml
	Distilled water	3.56 µl	7.12 ml	14.24 ml
	100X Phosphatase Inhibitors	40 µl	80 µl	160 µl
	TOTAL REQUIRED	**4.0 ml**	**8.0 ml**	**16.0 ml**
Complete Lysis Buffer	10 mM DTT	10.0 µl	30.0 µl	90.0 µl
	Lysis Buffer AM1	89.0 µl	267.0 µl	801.0 µl
	Protease Inhibitor Cocktail	1.0 µl	3.0 µl	9.0 µl
	TOTAL REQUIRED	**100.0 µl**	**300.0 µl**	**900.0 µl**

Step 1: Cell Collection

1. Aspirate media out of dish. Wash with 5 ml ice-cold PBS/Phosphatase Inhibitors. Aspirate solution out, and add 3 ml ice-cold PBS/Phosphatase Inhibitors. If working with suspension cells, pellet cells and wash with 3 ml ice-cold PBS/Phosphatase Inhibitors and proceed to step 3.
2. Remove cells from dish by gently scraping with cell lifter. Transfer cells to a pre-chilled 15 ml conical tube.
3. Centrifuge cell suspension for 5 minutes at 200 x *g* in a centrifuge pre-cooled at 4ºC.
4. Discard supernatant. Keep cell pellet on ice.

Step 2: Cell Lysis

1. Resuspend cell pellet in 300 µl Complete Lysis Buffer by pipetting up and down.
2. Incubate suspension for 10-30 minutes on ice on a rocking platform set at 150 rpm. Check a small sample under the microscope to verify that cells have been efficiently lysed to determine length of incubation. If the cells are not adequately lysed at this step, use an ice-cold Dounce homogenizer with a small-clearance (B) pestle to lyse the cells (refer to the Troubleshooting Guide in Section A of the Appendix, page 14, for further information).

Note: Do not proceed with the centrifugation step until the cells are sufficiently lysed.

4. Vortex 30 seconds at the highest setting. Centrifuge for 20 minutes at 14,000 x *g* in a microcentrifuge pre-cooled at 4°C. Transfer supernatant (whole-cell extract) into a pre-chilled microcentrifuge tube.
5. Aliquot and store at –80°C. Avoid freeze/thaw cycles.
   OPTIONAL: Save a fraction of the supernatant for determination of extraction efficiency by Western blot.

   Note: The presence of certain detergents or PMSF may interfere with the Bradford assay, thus perform a 1:50 or 1:250 dilution of your samples. Use the Complete Lysis Buffer as the blank with a similar dilution as the samples. Read the absorbance at 595 nm with a plate reader spectrophotometer (refer to the Appendix, Section A on page 13 for an example dilution series). As an alternative, try Active Motif's ProStain™ Protein Quantification Kit (Catalog No 15001), which offers greater sensitivity and resistance to many contaminating agents.

Protocol II: Fresh or Frozen Tissue

A. Preparation of Nuclear Extract from Tissue

Step 1: Tissue Homogenization

We recommend using only fresh tissue samples. If you have frozen tissue, we suggest you use the Whole-Cell Extract procedure (See page 12). However, nuclear fractionation with frozen tissue will produce quality nuclear extract, just at a lower yield. Samples should be kept on ice at all times. All steps are performed on ice or at 4°C with pre-cooled buffers and equipment. It can be generally estimated that there are 2 x 10^5 cells per milligram of tissue. However, we highly recommend that you perform an accurate quantitation of cell number for your specific tissue sample(s) prior to proceeding with the protocol.

Note: For Complete Lysis Buffer, remember to dilute 1 M DTT stock 1:100 to make 10 mM DTT.

Quick Chart for Preparing Buffers for Nuclear Extraction from Tissue

Reagents to Prepare	Components	1 gram	100 mg	50 mg
1X Hypotonic Buffer	10X Hypotonic Buffer	400 µl	40 µl	20 µl
(for tissue homogenization)	Protease Inhibitor Cocktail	40 µl	4 µl	2 µl
	100X Phosphatase Inhibitors	40 µl	4 µl	2 µl
	Distilled water	3.512 ml	351 µl	176 µl
	1 M DTT	4 µl	0.4 µl	0.2 µl
	Detergent	4 µl	0.4 µl	0.2 µl
	TOTAL REQUIRED	**4.0 ml**	**400 µl**	**200 µl**
1X Hypotonic Buffer	10X Hypotonic Buffer	50.0 µl	5.0 µl	2.5 µl
(for cytoplasmic fractionation)	Distilled water	450.0 µl	45.0 µl	22.5 µl
	TOTAL REQUIRED	**500.0 µl**	**50.0 µl**	**25.0 µl**
Complete Lysis Buffer	10 mM DTT	5 µl	0.5 µl	0.25 µl
	Lysis Buffer AM1	44.5 µl	4.45 µl	2.22 µl
	Protease Inhibitor Cocktail	0.5 µl	0.05 µl	0.025 µl
	TOTAL REQUIRED	**50.0 µl**	**5.0 µl**	**2.5 µl**
*(Optional) Detergent	**TOTAL REQUIRED**	**2.5 µl**	**0.5-1 µl**	**0.5-1 µl**

*The addition of Detergent to the nuclear pellet may help with solubility of proteins, specifically those tightly associated with membranes or chromatin.

Note: Volumes of reagents may need to optimized for each tissue.

1. Work quickly to preserve tissue during preparation.
2. For 1 gram of tissue, to obtain a single cell slurry, perform a tissue reduction.

   Fresh tissue: Dice tissue into 1-3 mm^3 pieces using a clean razor blade or scalpel. Keep tissue cold by performing this step in a cold ceramic mortar (mortar can be kept cold by submerging its base in liquid nitrogen). Collect pieces in a pre-chilled, clean Dounce homogenizer and dounce using a large-clearance (A) pestle.

   Frozen tissue: Crush desired amount of frozen tissue using a mortar and pestle with the base submerged in liquid nitrogen. Some liquid nitrogen can be placed in the mortar bowl along with the frozen tissue. The pestle can be cooled with liquid nitrogen.
3. This example protocol uses 1 gram of frozen tissue; please adjust the volumes for the amount of tissue used from the chart above. On ice, add 3 ml ice-cold 1X Hypotonic Buffer containing phosphatase and protease inhibitors supplemented with DTT and Detergent per gram of tissue and homogenize using the Dounce homogenizer with a large-clearance (A) pestle for approximately 20 strokes to disrupt tissue. Check under microscope to ensure that the cells are dissociated, continue homogenizing until you have a single cell slurry.
4. Incubate on ice for 15 minutes.
5. Centrifuge for 10 minutes at 850 x *g* at 4°C. Transfer the supernatant into a pre-chilled microcentrifuge tube.
   OPTIONAL: Save a fraction of the supernatant for determination of fractionation efficiency by Western blot.
6. At this point, the tissue is homogenized to a single cell slurry. However, most of the cells are not yet lysed. For cell lysis, continue the procedure with the cell pellet at Step 2, No. 1 of the protocol for Preparation of Nuclear Extract from Cells (page 7), based on a 100 mm tissue culture plate (8.8×10^6 cells).

B. Preparation of Whole-Cell Extract from Tissue

Quick Chart for Preparing Buffers for Whole-Cell Extraction from Tissue

Reagents to Prepare	Components	1 gram	100 mg	50 mg
Complete Lysis Buffer	10 mM DTT	4 µl	0.4 µl	0.2 µl
	Lysis Buffer	3.916 ml	392 µl	196 µl
	Protease Inhibitor Cocktail	40 µl	4 µl	2 µl
	100X Phosphatase Inhibitors	40 µl	4 µl	2 µl
	TOTAL REQUIRED	**4.0 ml**	**400 µl**	**200 µl**

Note: Volumes of reagents may need to optimized for each tissue.

1. For 1 gram of tissue, perform a tissue reduction by dicing tissue into 1-3 mm^3 pieces using a clean razor blade. Collect pieces in a pre-chilled 15 ml conical tube.
2. On ice, disrupt and homogenize tissue in 3 ml ice-cold Complete Lysis Buffer per gram of tissue with a Dounce homogenizer or a Polytron device. Maintain temperature at 4°C throughout all procedures. Incubate on ice for 30 minutes.

   Note: Frozen tissue can be sliced very thinly and thawed in this buffer. When using a mechanical homogenizer, begin homogenization at slow speeds until the tissue is broken into smaller pieces and then increase the speed to the maximum for 45-60 seconds. Avoid the generation of excess heat or foam.

3. Transfer to pre-chilled microcentrifuge tubes, centrifuge at 10,000 x *g* for 10 minutes at 4°C.
4. Transfer supernatants to new pre-chilled tubes and centrifuge again. Pool supernatants in the same tube. The supernatant contains the whole-cell lysate. Sometimes a longer centrifugation is necessary to obtain a clarified lysate.
5. Aliquot and store at –80°C. Avoid freeze/thaw cycles.
   OPTIONAL: Save a fraction of the supernatant for determination of extraction efficiency by Western blot.

   Note: The presence of certain detergents or PMSF may interfere with the Bradford assay, thus perform a 1:50 or 1:250 dilution of your samples. Use the Complete Lysis Buffer as the blank with a similar dilution as the samples. Read the absorbance at 595 nm with a plate reader spectrophotometer (refer to the Appendix, Section A on page 13 for an example dilution series). As an alternative, try Active Motif's ProStain™ Protein Quantification Kit (Catalog No 15001), which offers greater sensitivity and resistance to many contaminating agents.

Appendix

Section A. Example Dilution for Bradford Protein Quantitation

An example dilution series in preparation for the Bradford Assay is provided below.

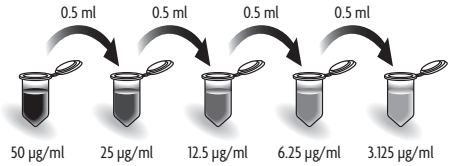
1. To measure the protein concentration utilizing the Bradford Assay, begin with a working stock of BSA (the example shown below uses working BSA stock at 5 mg/ml).

   Note: The DTT and protease inhibitors in the Complete Lysis Buffer require dilution before assaying for protein concentration. It is important to add the DTT and protease inhibitors to the lysis buffer before making the appropriate dilutions.

2. Dilute the Complete Lysis Buffer in preparation for the assay. The suggested dilutions are 1:50 or 1:250. Determine number of blank and standard wells you will be assaying and make enough diluted Lysis Buffer 1:50 or 1:250 stock to perform all dilutions. Dilute in water. Follow your Bradford Assay instructions for volume needed per well.

   Example: Lysis Buffer 1:50 stock = 30 µl Complete Lysis Buffer in 1470 µl H2O
   Lysis Buffer 1:250 stock = 6 µl Complete Lysis Buffer in 1494 µl H2O

3. Make up a 50 µg/ml BSA solution by adding 10 µl of the 5 mg/ml working BSA stock to 990 µl of Complete Lysis Buffer. Next, pipette 0.5 ml of Complete Lysis Buffer into the 4 remaining tubes. Use the 50 µg/ml BSA solution to prepare a dilution series as indicated below. The example standards below use the following concentrations: 50, 25, 12.5, 6.25, and 3.125 µg/ml. Note: The preceding range is provided as guidance, a broader range of values may be used. Refer to your Bradford Assay instructions for details. Mix tubes thoroughly before each transfer. Prepare Blank and samples. The 50 µg/ml standard is the high standard and the Blank (Complete Lysis Buffer alone) is 0.0 µg/ml.



   Example: 10 µl BSA (5 mg/ml) in 0.99 ml Complete Lysis Buffer 1:50 or 1:250 stock = 50 µg/ml
   Transfer 0.5 ml into 0.5 ml Complete Lysis Buffer 1:50 or 1:250 stock = 25 µg/ml
   Transfer 0.5 ml into 0.5 ml Complete Lysis Buffer 1:50 or 1:250 stock = 12.5 µg/ml
   Transfer 0.5 ml into 0.5 ml Complete Lysis Buffer 1:50 or 1:250 stock = 6.25 µg/ml
   Transfer 0.5 ml into 0.5 ml Complete Lysis Buffer 1:50 or 1:250 stock = 3.125 µg/ml

   Blank = Complete Lysis Buffer 1:50 or 1:250 stock
   Standards = dilute BSA into Complete Lysis Buffer 1:50 or 1:250 stock
   Samples = 1 µl sample diluted into 49 µl or 249 µl water

4. Analyze Blank, Standards and Samples by measuring the absorbance at 595 nm with a spectrophotometer according to your Bradford Assay instructions.

Section B. Troubleshooting Guide

Problem/question	Possible cause	Recommendation
Low protein concentration in cytoplasmic fraction	Volumes of extraction reagents not appropriate for given number of cells	Adjust volumes of reagents as indicated in the Quick Chart for Preparing Buffers.
	Plasma cell membrane not disrupted in cell pellet in Step 2, No. 1 of the Preparation of Nuclear Extract from Cells of Protocol I (page 7)	Gently pipette up and down to disrupt cell pellet. Monitor lysis under microscope. Use Dounce homogenizer with small-clearance (B) pestle.
Low protein concentration in nuclear fraction	Incorrect volume of Lysis Buffer or starting cell number	Decrease volume of Lysis Buffer or increase number of cells.
	Volumes of extraction reagents not appropriate for given number of cells	Adjust volumes of reagents as indicated in the Quick Chart for Preparing Buffers.
	Nuclear proteins lost in cytoplasmic fraction after rupture of nuclei	Monitor under microscope. Reduce vortex, centrifuge force and time in Step 2, Nos. 2 and 3 of the Preparation of Nuclear Extract from Cells section of Protocol I (page 7). Stop incubation when nuclei are released.
	Nuclear pellet not dispersed in Step 3, No. 1 of the Preparation of Nuclear Extract from Cells of Protocol I (page 7)	Vortex thoroughly to ensure nuclear lysis. Use the Dounce homogenizer if the pellet has trouble solubilizing.
No or low protein yield in either cytoplasmic or nuclear fractions	Cell type is not compatible with extraction procedure	Swelling and lysis conditions and reagents need to be optimized for this cell type.
	Overlysis	Nuclear proteins are present in cytoplasmic fraction. Reduce incubation time in Hypotonic Buffer. For frozen samples, cytoplasmic fraction will have proteins due to nuclear lysis during freeze/thaw.
	Incomplete lysis	Cytoplasmic proteins are present in the nuclear fraction. Check cells under a microscope after adding Detergent for presence of free nuclei indicative of efficient lysis. Alternatively, perform 10 strokes with a chilled Dounce homogenizer with a small-clearance (B) pestle to completely lyse cells.
Poor protein compartmentalization	Incomplete removal of cytoplasmic fraction	Make sure to remove all cytoplasmic fraction from the nuclear pellet before adding Lysis Buffer.
No or low protein activity	Collected proteins are degraded	Maintain low temperature requirements during procedure. Limit procedure time to a minimum and snap freeze aliquots right away. Add more or different protease inhibitors.
Nuclear pellet is viscous and will not resuspend	Incomplete dispersal of nuclear pellet	Vortex thoroughly to ensure nuclear lysis. Use the Dounce homogenizer if the pellet has trouble solubilizing. Certain cell types produce a viscous nuclear pellet and still produce efficient yield from the nuclear extraction.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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