

Cellular Fractionation

Lamond Lab Protocol 2007

More detailed protocol can be found here: <http://www.lamondlab.com/f7nucleolarprotocol.htm>

This protocol has been adapted to fractionate a variety of different cell types into cytoplasm, nucleoplasm and nucleoli. It uses a hypotonic buffer to swell the cells prior to dounce homogenization to break them open. A series of centrifugations over sucrose cushions follows, and nucleoli are released from purified nuclei by sonication. The solutions that you need are listed below.

We also include a simplified version of the protocol, which can be used to fractionate cells into cytoplasm and nuclei. This results in nuclei that are more pure than simply stopping the full protocol after the second step. If you are only fractionating into cytoplasm and nuclei, you will not need the S2 solution.

Solutions:

Buffer A (10 ml)

Hypotonic buffer that causes the cells to swell so that they can be efficiently broken open by dounce homogenizing.

Stock	Volume (ml)	mM (final)
1 M HEPES, pH 7.9	0.1	10
1 M MgCl ₂	0.015	1.5
2.5 M KCl	0.04	10
1 M DTT	0.005	0.5
dH ₂ O	Up to 10 ml	
Protease inhibitors	1 Mini EDTA-free COMPLETE tablet	

S1 (0.25 M Sucrose, 10 mM MgCl₂) 20 ml

Sucrose buffer used to fractionate nuclei.

Stock	Volume (ml)	mM (final)
2.5 M Sucrose	1.96	0.25
1 M MgCl ₂	0.2	10
dH ₂ O	Up to 20 ml	
Protease inhibitors	1 EDTA-free COMPLETE tablet	

S2 (0.35 M Sucrose, 0.5 mM MgCl₂) 40 ml

Sucrose buffer used to sonicate nuclei and release nucleoli. The Mg level is critical, so remember to always use EDTA-free protease inhibitor tablets!.

Stock	Volume (ml)	mM (final)
2.5 M Sucrose	5.5	0.35
1 M MgCl ₂	0.02	0.5
dH ₂ O	Up to 40 ml	
Protease inhibitors	2 EDTA-free COMPLETE tablets	

S3 (0.88 M Sucrose, 0.5 mM MgCl₂) 20 ml

Sucrose buffer used to fractionate nucleoli.

Stock	Volume (ml)	mM (final)
2.5 M Sucrose	6.9	0.88
1 M MgCl ₂	0.01	0.5
dH ₂ O	Up to 20 ml	
Protease inhibitors	1 EDTA-free COMPLETE tablet	

RIPA Buffer (10 ml)

Detergent buffer used to solubilize proteins, either in whole cells or in purified cytoplasmic, nucleoplasmic or nucleolar fractions.

Stock	Volume (ml)	mM (final)
1M Tris, pH 7.5	0.5	50
5 M NaCl	0.3	150
10% NP-40	1	1%
10% Deoxycholate	0.5	0.5%
Protease inhibitors	1 Mini EDTA-free COMPLETE tablet	

Starting Material:

We normally use between 5 and 15 x 14 cm dishes of confluent cells for this fractionation.

Fractionation:

- Trypsinize cells, pellet at 1000 rpm for 4 min, wash with PBS and pellet again at 1000 rpm for 4 min. Resuspend in 5 ml of ice-cold Buffer A and keep on ice for 5 min. Break open cells to release nuclei using a pre-chilled 7 ml Dounce homogenizer (10 strokes with a tight pestle).
- Centrifuge Dounced cells at 228 g (1000 rpm, Beckman GS-6, GH-3.8 rotor) for 5 min at 4C to pellet nuclei and other fragments. The supernatant can be retained as the [cytoplasmic fraction](#).
- Resuspend the nuclear pellet in 3 ml of S1 and layer over a 3 ml cushion of S2. Centrifuge at 1430 g (2500 rpm, Beckman GS-6, GH-3.8 rotor) for 5 min at 4C. This step results in a cleaner nuclear pellet.
- Resuspend the clean, pelleted nuclei in 3 ml of S2 and sonicate for 6 x 10 sec (with 10 sec rest between each sonication) at full power in an ice bath to prevent overheating of the sample. Check the sonicate using phase contrast microscopy to ensure that no intact nuclei remain, and that the nucleoli are readily observable as dense, refractile bodies. If you still see intact nuclei, continue to sonicate (adding 2 x 10 sec steps and checking again).
- Layer the sonicated sample over 3 ml of S3 and centrifuge at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 10 min at 4C. The pellet contains the nucleoli and the supernatant can be retained as the [nucleoplasmic fraction](#).
- Wash the [nucleoli](#) by resuspending in 0.5 ml of S2 and centrifuge at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 5 min at 4C. They can be stored in any volume of this buffer at -80C by snap-freezing them in liquid nitrogen. Before using them they can be spun out of the buffer again with the same spin.

Fractionation of only cytoplasm and nuclei:

- Trypsinize cells, pellet at 1000 rpm for 4 min, wash with PBS and pellet again at 1000 rpm for 4 min. Resuspend in 5 ml of ice-cold Buffer A and keep on ice for 5 min. Break open cells to release nuclei using a pre-chilled Dounce homogenizer (20 strokes with a tight pestle).
- Centrifuge Dounced cells at 228 g (1000 rpm, Beckman GS-6, GH-3.8 rotor) for 5 min at 4C to pellet nuclei and other fragments. The supernatant can be retained as the [cytoplasmic fraction](#).
- Resuspend the nuclear pellet in 3 ml of S1 and *layer over a 3 ml cushion of S3*. Centrifuge at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 10 min at 4C. This step results in a cleaner [nuclear pellet](#).

Preparing lysates from cellular fractions:

We normally use RIPA buffer to prepare lysates from our cellular fractions. Pelleted nuclei and nucleoli can be resuspended directly in 1X RIPA buffer, but cytoplasmic and nucleoplasmic fractions will need to have 5X RIPA buffer added to them to bring the final buffer as close to RIPA as possible. There will still be some small amount of sucrose present in these lysates, but that can be minimized by increasing the final lysate volume. Alternately, these fractions can be dialyzed into 1X RIPA buffer.

Full Fractionation (Cytoplasm, Nucleoplasm and Nucleoli)

Cytoplasmic Lysate:

The fractionation detailed above results in a 4 ml cytoplasmic fraction. We add 1 ml of 5X RIPA buffer to this, mix well and then centrifuge at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 10 min at 4C to pellet any solids.

Nucleoplasmic Lysate:

The fractionation detailed above results in a 6 ml nucleoplasmic fraction. We add 2 ml of 5X RIPA buffer and 2 ml of dH₂O to this, mix well and centrifuge at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 10 min at 4C to pellet any solids.

Nucleolar Lysate:

The fractionation detailed above results in a very small nucleolar pellet, which we resuspend in 1 ml of 1X RIPA buffer and then sonicate 5 x 10 sec on ice to ensure release of as many nucleolar proteins as possible.

Simple Fractionation (Cytoplasm and Nuclei)

Cytoplasmic Lysate:

The fractionation detailed above results in a 4 ml cytoplasmic fraction. We add 1 ml of 5X RIPA buffer to this, mix well and then centrifuge at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 10 min at 4C to pellet any solids.

Nuclear Lysate:

The fractionation detailed above result in nuclear pellet of approximately 0.5 ml volume. We add 5 ml of 1X RIPA buffer to this, mix well and then sonicate 5 x 10 sec on ice to ensure release of as many nuclear proteins as possible (and to shear DNA). We then centrifuge the lysate at 2800 g (3500 rpm, Beckman GS-6, GH-3.8 rotor) for 10 min at 4C to pellet any solids.

Preparing larger volumes of buffers:

Buffer A (50 ml)

Stock	Volume (ml)
1 M HEPES, pH 7.9	0.5
1 M MgCl ₂	0.075
2.5 M KCl	0.2
1 M DTT	0.025
Protease inhibitors	1 EDTA-free COMPLETE tablet

S1 (0.25 M Sucrose, 10 mM MgCl₂) 50 ml

Stock	Volume (ml)
2.5 M Sucrose	4.9
1 M MgCl ₂	0.5
dH ₂ O	Up to 50 ml
Protease inhibitors	2 EDTA-free COMPLETE tablets

S2 (0.35 M Sucrose, 0.5 mM MgCl₂) 50 ml

Stock	Volume (ml)
2.5 M Sucrose	6.9
1 M MgCl ₂	0.025
dH ₂ O	Up to 50 ml
Protease inhibitors	2 EDTA-free COMPLETE tablets

S3 (0.88 M Sucrose, 0.5 mM MgCl₂) 50 ml

Stock	Volume (ml)
2.5 M Sucrose	17.3
1 M MgCl ₂	0.025
dH ₂ O	Up to 50 ml
Protease inhibitors	2 EDTA-free COMPLETE tablets

RIPA Buffer (50 ml)

Stock	Volume (ml)
1M Tris, pH 7.5	2.5
5 M NaCl	1.5
10% NP-40	5
10% Deoxycholate	2.5
Protease inhibitors	2 EDTA-free COMPLETE tablets

