Product Information

Lysosome Isolation Kit

For enrichment of lysosomes from tissues and packed cells, sufficient for 25 g (tissue), sufficient for 20 mL (packed cells)

LYSISO1

Product Description

Lysosomes are organelles ubiquitously distributed in most eukaryotic cells. They are spherical particles with a diameter of 0.5-1.5 microns and a low pH environment (pH ~5.0). Lysosomes contain many acid hydrolases that participate in cellular protein degradation. Lysosomes also contain lipases, polysaccharidases, and nucleases. Deficiencies in some of these enzymes lead to specific lysosomal storage diseases,[1,2] such as Tay-Sachs, Gaucher, and Hunter diseases. Lysosomes also contribute to maintaining cellular homeostasis. Thus, lysosomal malfunctions directly impact cell behavior and fate.[3] Lysosomes may also be involved in other cellular processes such as albinism[4] and aging.[5,6]

The Lysosome Isolation Kit provides a procedure to isolate an enriched lysosomal fraction from animal tissues and cultured cells by differential centrifugation followed by density gradient centrifugation and/or calcium precipitation. The presence of lysosomes can be determined by measuring either:

- Acid phosphatase activity, with the Acid Phosphatase Assay Kit (Cat. No. CS0740), or
- β-*N*-Acetylglucosaminidase activity, with the β-*N*-Acetylglucosaminidase Assay Kit (Cat. No. CS0780)

These two enzymes are considered lysosomal markers, which will show slightly different patterns on an OptiPrep™ density gradient.[7] Separation from other organelles can be measured using the appropriate marker detection kits. (See the "Additional equipment and reagents required for measuring enzyme activities" section.)

Several theses[8] and dissertations[9-19] have cited use of LYSISO1 in their protocols.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

After receiving the kit, the Protease Inhibitor Cocktail (Component P8340) should be stored at −20 °C and the OptiPrep™ Density Gradient Medium (Component O3028) should be stored at room temperature.

All the other components in this kit should be stored at 2-8 °C. The components are stable for 24 months when stored unopened.

Components

The kit is sufficient for the preparation of lysosomes from either 25 g of tissue or 20 mL of packed cells.

- Extraction Buffer 5× (Component E1156): 100 mL
- OptiPrep™ Dilution Buffer 20× (Component O4889): 20 mL
- Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts (Component P8340): 5 mL
- OptiPrep™ Density Gradient Medium, 60% (w/v) solution of iodixanol in water (Component O3028): 100 mL
- Neutral Red Reagent (Component N2537): 1 mL
- Calcium Chloride Solution, 2.5 M $CaCl_2$ solution (Component C2052): 1 mL
- Sucrose Solution, 2.3 M (Component S4189): 25 mL

Reagents and Equipment Required

(Not provided)

Example Cat. Nos. are given where appropriate.

General Additional Reagents and Equipment

- Centrifuge with SS-34 head or equivalent
- Ultracentrifuge with SW50.1 head or equivalent and 5 mL tubes
- Microcentrifuge
- Microcentrifuge tubes
- Ultrapure water
- Dulbecco's Phosphate Buffered Saline (DPBS), such as Cat. No. D8537)
- Pasteur pipettes
- Finntip® Flex 1,000 pipette tips (Cat. No. Z677914)
- Bradford Reagent, for protein measurements (Cat. No. B6916)

Equipment required for preparation of lysosomes from tissue extracts

- Homogenizers
- For initial homogenization: Tissue homogenizer with S25N 18G head or equivalent
- Overhead electric motor together with Potter-Elvehjem PTFE pestle in glass tube homogenizer: 3 mL (Cat. No. P7734) or 8 mL (Cat. No. P7859)
- Scalpel, forceps, and glass plate
- 40 mL Polypropylene (PP) centrifuge tubes

Equipment and reagents required for preparation of lysosomes from cell culture extracts

- Trypsin-EDTA (for removal of adherent cells from vessel surface)
- Trypan Blue solution (Cat. No. T8154)
- Refrigerated centrifuge
- Adapter for microcentrifuge tubes
- 14 mL centrifuge tubes with adapter for SS-34 head
- Homogenizers:
- 7 mL Dounce glass tissue grinder (Cat. No. T0566) with small clearance pestle (Cat. No. P1235) for cell culture samples
- Pellet pestle
- Motor for pellet pestle (Cat. No. Z359971)

Additional equipment and reagents required for measuring enzyme activities

- Acid Phosphatase Assay Kit (Cat. No. CS0740): Acid phosphatase is a lysosomal marker.
- β-*N*-Acetylglucosaminidase Assay Kit (Cat. No. CS0780)
- Cytochrome c Oxidase Assay Kit (Catalog Number CYTOCOX1): Cytochrome c oxidase is a mitochondrial marker.
- Cytochrome c Reductase Assay Kit (Cat. No. CY0100): Cytochrome c reductase is an endoplasmic reticulum (ER) marker.
- Catalase Assay Kit (Cat. No. CAT100): Catalase is a peroxisomal marker.

  Note: To monitor the degree of purification following separation on an OptiPrep™ density gradient, catalase activity must be determined by the colorimetric method, since OptiPrep™ Density Gradient Medium interferes with the UV method.
- Spectrophotometer with a 1 mL cuvette, to measure enzyme activities

Preparation Instructions

It is recommended to use ultrapure water (17 MΩ·cm or equivalent) when preparing the reagents.

250 mM Calcium Chloride Solution

Dilute an aliquot of the 2.5 M calcium chloride solution (Component C2052) 10-fold with ultrapure water.

1× Extraction Buffer

1. Dilute an aliquot of the Extraction Buffer 5× (Component E1156) 5-fold with ultrapure water. Keep the diluted Extraction Buffer at 2-8 °C until use.
2. Just before use, add the Protease Inhibitor Cocktail for mammalian cell and tissue extracts (Component P8340) to the diluted Extraction Buffer at a final concentration of 1% (v/v). This diluted Extraction Buffer with the 1% (v/v) Protease Inhibitor Cocktail is the 1× Extraction Buffer.
3. Suggested volumes of 1× Extraction Buffer:

   For tissue extracts

   3.1. Use a minimal tissue weight of 4 g.

   3.2. Prepare 25 mL of buffer.

   For cell culture extracts

   3.3. Use a minimum of 2-3 × 10^8 cells.

   3.4. Prepare 10 mL of buffer.

1× OptiPrep™ Dilution Buffer

1. Dilute an aliquot of the OptiPrep™ Dilution Buffer 20× (Component O4889) 20-fold with ultrapure water.
2. Keep the 1× OptiPrep™ Dilution Buffer at 2-8 °C until use.
3. Suggested volumes of 1× OptiPrep™ Dilution Buffer:
   3.1. For tissue extracts from 4 g of tissue: 40 mL
   3.2. For cell culture extracts from at least 2×10^8 cells: 30 mL

OptiPrep™ Density Gradient Medium Solutions

- Prepare 10 mL of each OptiPrep™ Density Gradient Medium Solution (see Table 1).
- The OptiPrep™ Density Gradient Medium (Component O3028) supplied with the kit is a 60% (w/v) solution in water.
- Table 1 shows the final concentration of each OptiPrep™ Density Gradient Medium Solution after dilution.
- The diluted solutions need to be osmotically balanced with sucrose to ~290 mOsm.
- These OptiPrep™ Density Gradient Medium Solutions may be kept at 2-8 °C for up to 4 weeks, if prepared aseptically.

Table 1. Preparation of OptiPrep™ Density Gradient Medium Solutions

OptiPrep™ (%)	OptiPrep™ (mL)	OptiPrep™ Dilution Buffer (mL)	2.3 M Sucrose (mL)	mOsm*
27	4.5	4.9	0.6	312
22.5	3.75	5.63	0.62	304
19	3.17	6.19	0.64	298
16	2.67	6.68	0.65	280
12	2	7.29	0.71	300
8	1.33	7.9	0.77	282

* These values are representative only. It is recommended to determine the actual values experimentally (the target value should be ~290 mOsm).

Procedure

A crude lysosomal fraction can be prepared using a simple method of homogenization followed by differential centrifugation.

The serial centrifugations include:

- Low speed centrifugation (1,000 × g)
- Medium speed centrifugation (20,000 × g)

The crude lysosomal fraction (CLF) is obtained after removal of nuclei, cell debris, and fat by the serial centrifugations. The CLF pellet is the starting material for the further preparation of purified lysosomes.

Lysosomes may be further purified on a multi-layered step gradient of osmotically balanced OptiPrep™[20] and/or purified by precipitation of rough ER and mitochondria with calcium ions.

A flow chart for the various preparations of lysosomes is shown in Appendix I.

Preparation of Crude Lysosomal Fraction (CLF)

A. From Animal Tissue (~4 g of tissue)

- Perform the whole procedure at 2-8 °C.
- All the solutions and equipment should be pre-cooled before use.
- Homogenize the samples using a homogenizer with a S25N 18G head.

1. Use a fresh tissue sample from an animal that was starved overnight and sacrificed the next morning.
2. Wash the tissue sample three times with 10-15 mL of ice-cold PBS:
   2.1. Place the tissue sample in a dish.
   2.2. Shake gently for a few minutes.
   2.3. Remove the PBS.
   2.4. Place the tissue on a paper towel, to absorb excess liquid and blood clots, if present.
   2.5. Cut the tissue into small pieces (1.5-2 cm).
   2.6. Repeat the wash step.
3. Blot the tissue on a paper towel. Weigh.
4. Cut the tissue on a glass plate with the aid of a scalpel into small slices (0.3-0.5 cm).
   4.1. Transfer the slices into a 40 mL polypropylene (PP) centrifuge tube.
   4.2. Add 4 volumes of the 1× Extraction Buffer per gram of tissue (such as 16 mL per 4 g).
   4.3. Homogenize the sample as follows:
   - Homogenization at 8,000 rpm for 5 seconds,
   - Followed by homogenization at 9,500 rpm for two additional 5-second periods.
5. Wash the homogenizer head with 1 mL of the 1× Extraction Buffer.
   5.1. Add to the previous homogenate.
   5.2. Keep the homogenate on ice.
6. Centrifuge the homogenate at 1,000 × g for 10 minutes at 2-8 °C.
   6.1. Remove the floating fat layer by careful aspiration.
   6.2. Transfer the supernatant to another centrifuge tube using a pipette.

7. Rehomogenize the pellet in 2 volumes of 1× Extraction Buffer for 5 seconds at 9,500 rpm.
   7.1. Repeat Step 6.
   7.2. Pool the supernatant from Step 7.1 with the supernatant from Step 6. This is defined as the 1,000 × g supernatant.

   Note: To monitor the degree of purification following the different centrifugation steps, it is recommended to save a sample (~200 µL) of the 1,000 × g supernatant for subsequent assays.
8. Discard the pellet, which contains nuclei and other cell debris.
9. Centrifuge at 20,000 × g for 20 minutes at 2-8 °C. Remove the floating fat layer and the supernatant by aspiration.
10. Resuspend the pellet in a minimal volume of 1× Extraction Buffer.
    - It is recommended to use 0.8 mL per gram of original tissue (such as 3.2 mL per 4 g).
    - This material is the Crude Lysosomal Fraction (CLF) and contains a mixture of light mitochondria, lysosomes, peroxisomes, and endoplasmic reticulum.
    - **Note**: For further purification, it is recommended to continue directly to the "Further Purification of CLF" section. If necessary, the CLF may be stored overnight at 2-8 °C (**do not freeze**) before continuing.

B. From cultured cells (~3 × 10^8 cells)

- Perform the whole procedure at 2-8 °C.
- All the solutions and equipment should be pre-cooled before use.
- Homogenize the samples using a 7 mL Dounce glass tissue grinder (Cat. No. T0566) with a small clearance pestle (Cat. No. P1235).
- **Note**: This procedure requires relatively large amounts of cells, on the order of 1.5-3 mL packed cell volume, representing at least 2-3 × 10^8 cells.

1. Grow the cells to ~90% confluency.

2a. For adherent cells:

- Trypsinize the cells.
- Add growth medium with 10% fetal calf serum.
- Centrifuge the cells for 5 minutes at 600 × g.

2b. For cells in suspension:

- Perform the centrifugation only.
- Discard the supernatant.

3. Wash the cells:
   3.1. Resuspend the cells in ice cold PBS.
   3.2. Count the cells.
   3.3. Centrifuge for 5 minutes at 600 × g at 2-8 °C.
   3.4. Discard the supernatant.
4. Repeat the wash step once again (without the cell count). Discard the supernatant. The packed cell volume (PCV) should be 1.5-3 mL.
5. Add 2.7 PCV of 1× Extraction Buffer. Vortex to achieve an even suspension.
6. Break the cells in a 7 mL Dounce homogenizer using Pestle B (small clearance). This may require dividing the fraction into two portions.
7. After every 5 strokes with the pestle, check the cells under a microscope using Trypan Blue solution staining, to ascertain the degree of breakage.
   - Normally 15-25 strokes will suffice to achieve 80-85% of breakage.
   - Do not try to achieve higher levels, as this will lead to severe breakage of the lysosomes.
8. Centrifuge the sample at 1,000 × g for 10 minutes.
9. Transfer the supernatant to a new centrifuge tube. Keep a sample (100-200 µL) of the supernatant for subsequent assays.
10. Centrifuge the sample at 20,000 × g for 20 minutes in microcentrifuge tubes.
11. Remove the supernatant liquid. Collect the pellet in a minimal volume of 1× Extraction Buffer (~0.4 mL per 10^8 cells).
12. Suspend the pellet well in a single microcentrifuge tube by using a pellet pestle (see Equipment). This material is a Crude Lysosomal Fraction (CLF), containing a mixture of mitochondria, lysosomes, peroxisomes, and endoplasmic reticulum.

    Note: For further purification, it is recommended to continue directly to Section II ("Further Purification of CLF"). If necessary, the CLF may be stored overnight at 2-8 °C (**do not freeze**) before continuing.

Further Purification of CLF

To enrich further the lysosomes in the CLF, the organelles should be separated from each other. Three options are available for further purification. Common to all the options is dilution of the CLF to a solution that contains 19% OptiPrep™ Density Gradient Medium Solution with a protein concentration of either:

- 5-10 mg of protein/mL for tissues, or
- 0.5–1.0 mg of protein/mL for cell culture extracts

This solution is defined as the Diluted OptiPrep™ Fraction (DOF). The purification options are as follows:

Option A: Separation of the DOF by density gradient centrifugation (150,000 × g for 4 hours) on a multi-step OptiPrep™ gradient.

- This method allows separation into heavy and light lysosomes with a relatively high yield (>50%).

Option B: Further purification of the fractions obtained from Option A by addition of $CaCl_2$ to a final concentration of 8 mM and low speed (5,000 × g for 15 minutes) centrifugation.

- This method will precipitate the rough endoplasmic reticulum and any mitochondria that are in the fraction.
- There will be a partial loss of lysosome yield with this step.

Option C: Addition of $CaCl_2$ to the DOF to a final concentration of 8 mM and low speed (5,000 × g for 15 minutes) centrifugation.

- This method is very quick and allows purification of the sample without the use of an ultracentrifuge.
- However, the yield is low (<25%).

Option A: Isolation of lysosomes on a density gradient

The procedure is for a 5 mL ultracentrifuge tube.

1. Dilute the CLF (see Table 2):
   1.1. Add the components in order from left to right, mixing at each stage.
   1.2. If the sample is not diluted, it will tend to drop into a lower density layer. This solution is defined as the Diluted OptiPrep™ Fraction (DOF).

Table 2. Preparation of 19% Diluted OptiPrep™ Fraction (DOF) for Loading on the Gradient.

Sample	OptiPrep™ (O3028) (mL)	OptiPrep™ Dilution Buffer (mL)	2.3 M Sucrose (mL)	CLF (mL)	mOsm*
Liver	0.505	0.65	0.03	0.40	280
Jurkat	0.505	0.275	0.00	0.80	300

* These values are representative only. It is recommended to determine the actual values experimentally (the target value should be ~290 mOsm).

2. Build a step gradient (see Table 3) with 27% OptiPrep™ Density Gradient Medium Solution at the bottom and 8% OptiPrep™ Density Gradient Medium Solution at the top of the tube. Use the OptiPrep™ Density Gradient Medium Solutions described in Table 1 (Preparation Instructions).

Table 3. OptiPrep™ Gradient

OptiPrep™ Solution (%)	Volume (mL)	Sample
27	0.8	
22.5	1.0	
19	1.0	Prepared DOF (Step 1)
16	1.0	
12	0.9	
8	0.3	

3. Balance two tubes against each other.
4. Centrifuge 4 hours at 150,000 × g in an SW50.1 rotor.
   4.1. Brake to 800 rpm.
   4.2. Then let stop without brake.

   Note: If an ultracentrifuge is not available, it is possible to centrifuge the density gradient in 8 mL tubes in a superspeed centrifuge for 12 hours at 45,000 × g (19,400 rpm in an SS-34 head). A similar separation will be observed.
5. The tube will show multiple bands floating in the gradient. Note that samples from different sources will give different patterns of separation on the OptiPrep™ density gradient.
6. Withdraw fractions of 0.5-0.7 mL using extra-long tips (such as Finntip® Flex 1,000), starting from the top of the gradient. Number the fractions.
7. Assay the purified fractions for:
   7.1. Protein concentration, using the Bradford method: 20 µL per test with 20-fold dilution
   7.2. Acid phosphatase activity: 40-80 µL per test without dilution
   7.3. β-*N*-Acetylglucosaminidase activity:
   - For cells: 10 µL per test without dilution
   - For tissues: 2.5-5 µL per test with 2-fold to 4-fold dilution

   7.4. Cytochrome c oxidase activity: 2.5-25 µL per test with 5-fold dilution
   7.5. Cytochrome c reductase activity: 25 µL per test without dilution

7.6. Catalase activity:

- For cell extracts: 10 μL per test with 2-fold dilution
- For tissues: 5 μL per test with 10-fold to 100-fold dilution

Note: To monitor the degree of purification following the separation on an OptiPrep™ density gradient, catalase activity must be determined by the colorimetric method, since OptiPrep™ Density Gradient Medium interferes with the UV method.

8. Various assays may be performed on the 1,000 × g supernatant from either of these following steps in the "Preparation of Crude Lysosomal Fraction (CLF)" procedure:
   - Part A (From Animal Tissue), Step 7, or
   - Part B (From Cultured Cells), Step 9

8.1. Protein concentration (Bradford method):

- For tissues: 10-40 μL per test with 100-fold dilution
- For cell extracts: 10-40 μL per test with 10-fold dilution

8.2. Acid phosphatase activity (without dilution):

- For tissues: 5-10 μL per test
- For cell extracts: 10-40 μL per test

8.3. β-*N*-Acetylglucosaminidase activity (without dilution):

- For tissues: 10-20 μL per test
- For cells: 10 μL per test

8.4. Cytochrome c oxidase activity:

- For tissues: 5-10 μL per test with 20-fold dilution
- For cell extracts: 50 μL per test with 2-fold dilution

8.5. Cytochrome c reductase activity (without dilution):

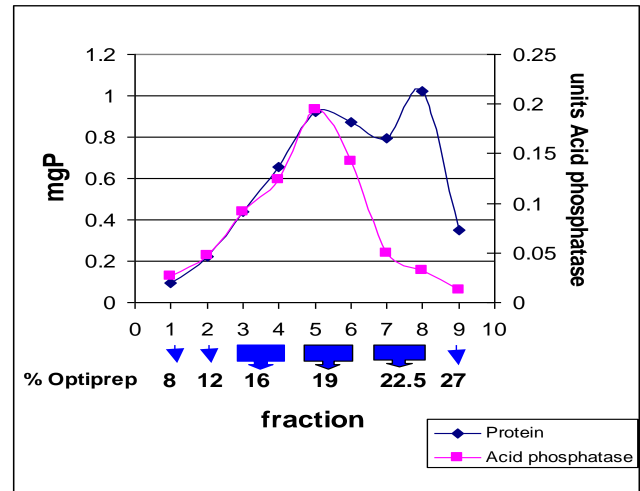
- For tissues: 2.5-5 μL per test
- For cell extracts: 50 μL per test

8.6. Catalase activity:

- For cell extracts: 10 μL per test with 2-3 fold dilution
- For tissues: 2.5-5 μL per test with 100-fold dilution
- **Note**: The 1,000 × g supernatant, which does not contain the OptiPrep™ Density Gradient Medium, may be assayed for catalase activity using either the colorimetric or UV method.
- However, for direct comparison of catalase activity in the 1,000 × g supernatant to purified fractions separated on the OptiPrep™ density gradient, the colorimetric method must be used in all assays.

This procedure will result in the separation of the organelles (see Figure 1).

Figure 1. Separation of Lysosomes from Rat Liver on an OptiPrep™ Gradient



8 mg of protein of the CLF were used to prepare the DOF and then placed on a 5 mL step gradient ("Further Purification of CLF", Option A, Step 2). The blue arrows show the approximate position of the OptiPrep™ gradient.

Alternative preparation of DOF

To achieve good separation of the lysosomes, it is possible to change the initial OptiPrep™ Density Gradient Medium concentration in the sample. This may lead to slightly different contamination with other organelles. The preparation of a sample (DOF) with a 16% OptiPrep™ Density Gradient Medium concentration, a recommended alternative, is shown in Table 4.

Table 4. Preparation of 16% Diluted OptiPrep™ Fraction (DOF) for Loading on the Gradient

Sample	OptiPrep™ (O3028) (mL)	Optiprep™ Dilution Buffer (mL)	2.3 M Sucrose (mL)	CLF (mL)	mOsm
Liver	0.425	0.72	0.04	0.4	292

Option B: Further purification of density gradient fractions with the aid of $CaCl_2$

1. Prepare a 250 mM Calcium Chloride ($CaCl_2$) Solution as described under the Preparation Instructions.
2. Take a 200 μL aliquot of each fraction from the OptiPrep™ gradient. Add the 250 mM $CaCl_2$ Solution to a final concentration of 8 mM (6.5 μL per 200 μL of sample). Mix.
3. Incubate on ice for 15 minutes. Then centrifuge at 5,000 × g for 15 minutes in a microcentrifuge.
4. Transfer the supernatant liquid into a fresh tube. The majority of the lysosomes (>50%) will remain in the supernatant.

Option C: Direct addition of $CaCl_2$ to the DOF

If no ultracentrifuge is available and there is only a limited amount of material, it is possible to remove contaminating mitochondria and endoplasmic reticulum by direct addition of the 250 mM $CaCl_2$ Solution (to a final concentration of 8 mM) to the sample prepared for the OptiPrep™ gradient (DOF, see Table 2). That will give a lower yield of purified lysosomes than the gradient purification (25% rather than >50%) but is a much simpler procedure.

Storage of crude and purified lysosomes

- The CLF may be kept overnight at 2-8 °C and then separated the next day on a density gradient.
- Also, the purified lysosomes may be kept for up to 24 hours with little degradation.
- If a Western blot of specific proteins in the lysosome is desired, it is advisable to add the sample buffer immediately after preparation of the purified fraction and then to freeze the sample.

Measurement of Intactness of the Lysosomes

The intactness of the lysosomes can be assessed using the dye Neutral Red. The absorbance maximum of the Neutral Red dye shifts from 460 nm to 510 nm in the acidic pH conditions found in the lysosome. The Neutral Red dye will concentrate in the lysosomes, where it binds by electrostatic hydrophobic bonds with anionic sites in the lysosomal matrix.[21,22] The uptake may be followed by a spectrophotometer in real time.

1. Set up a spectrophotometer on "Time Drive", using a program for 3 minutes at the two wavelengths 460 nm and 510 nm.
2. Place up to 990 μL of 1× Extraction Buffer in a 1 mL disposable cuvette. The volume is dependent on the amount of sample to be added to the cuvette.
3. Add 2.5 μL of Neutral Red Reagent (Component N2537). Mix well by inversion.
4. Add the requisite number of lysosomes (30-120 μg protein, Options A, B, or C) up to 100 μL volume. Start the program.
5. Obtain the values for each wavelength in the time period from 5-65 seconds.

Note: If a spectrometer is not available with a time drive option and multiple wavelength readings, the same sample can be measured at 460 nm and 510 nm consecutively.

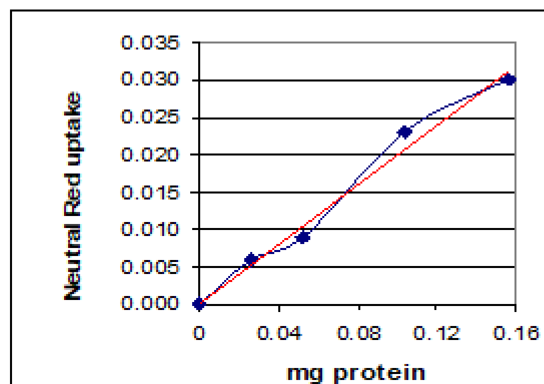
Calculation

Since the dye absorbance at 460 nm changes very little during uptake, any change in this value is probably a result of fluctuations due to the biological material added. This effect can be removed by subtracting the value obtained at 460 nm from that obtained at 510 nm. The value desired is:

Neutral Red Uptake = $(\Delta A_{510} / \text{min}) - (\Delta A_{460} / \text{min})$

Figure 2 shows an example of the linearity of the dye uptake with increasing amounts of lysosomal protein.

Figure 2. Linearity of Dye Uptake with Increasing Amounts of Lysosomal Protein



The suspended 20,000 × g pellet, from either (a) "Part A (From Animal Tissue)", Step 10 or (b) "Part B (From Cultured Cells)", Step 11, of the "Preparation of Crude Lysosomal Fraction (CLF)" Procedures, was added to the mixture at varying amounts of protein. The uptake was linear from 25-150 μg of protein.

References

1. Grabowski, G.A., and Hopkin, R.J., *Annu. Rev. Genomics Hum. Genet*., **4**, 403-436 (2003).
2. Wilcox, W.R., *J. Pediatr*., **144(5 Suppl)**, S3-S14 (2004).
3. Castino, R. *et al*., *J. Mol. Recognit*., **16(5)**, 337-348 (2003).

4. Stinchcombe, J. *et al*., *Science*, **305(5680)**, 55-59 (2004).
5. Nakamura, Y. *et al*., *Neurosci. Lett*., **97(1-2)**, 215-220 (1989).
6. Kenessey, A. *et al*., *J. Neurosci. Res*., **23(4)**, 454-456 (1989).
7. Gottwik, M.G. *et al*., *J. Clin. Invest*., **56(4)**, 914-923 (1975).
8. Trivedi, Purvi C., "Nutrient Regulation of Autophagy in the Heart". Dalhousie University, M.Sc. thesis, p. 32 (2016).
9. Andreyeva, Aksana, "Neural recognition molecule CHL1: regulation of the activity of the trimeric protein complex Csp/Hsc70/Sgt and synaptic vesicle recycling in *Mus musculus* (Linnaeus, 1758)". Universität Hamburg, Dr. rer. nat. dissertation, pp. 37, 49 (2008).
10. Xiao, Meifang, "Neural cell adhesion molecule NCAM modulates dopamine-related behavior by regulating dopamine D2 receptor internalization in mice (*Mus musculus* Linnaeus, 1758)". Universität Hamburg, Dr. rer. nat. dissertation, p. 50 (2009).
11. Neves, Juliana de Carvalho, "Regulação de receptores de IGF e PDGF na musculatura esquelética de camundongos com deficiência de neuraminidase 1" ("Regulation of IGF and PDGF receptors in skeletal muscle of neuraminidase 1-deficient mice"). Universidade de São Paulo, Ph.D. dissertation, p. 24 (2011).
12. González-Leal, Iris J., "Roles of Cathepsins B and L in the Th1/Th2 polarization by dendritic cells". Julius-Maximilians Universität Würzburg, Dr. rer. nat. dissertation, p. 66 (2014).
13. Balakrishnan, Vijaya, "Regulation of Autophagy and Lysosome Integrity by Plac8 in Cancer". University of Rochester, Ph.D. dissertation, p. 134 (2015).
14. Massner, Christoph, "Genetically controlled iron uptake and storage in mammalian cells for magnetic resonance imaging and magnetic actuation". Technischen Universität München, Dr. rer. nat. dissertation, pp. 38, 121 (2018).
15. Martinelli, Silvia, "Effect of stress on protein homeostasis mediated by FKBP51 as a possible mechanism underlying stress-related disorders". Ludwig-Maximilians-Universität München, Dr. rer. nat. dissertation, p. 22 (2019).
16. Saffi, Golam Tanjib, "Understanding PIKfyve Control of Lysosome Dynamics". Ryerson University, Ph.D. dissertation, p. 72 (2019).
17. Ultimo, Amelia, "Design and development of new bio-gated nanodevices for targeted controlled drug release". Universitat Politècnica de València, Ph.D. dissertation, pp. 52, 61, 66, 84, 90, 110, 112 (2019).
18. Haberger, Vanessa, "Characterizing the turnover of viral nonstructural proteins of the hepatitis C virus". Johannes Gutenberg-Universität Mainz, Dr. rer. nat. dissertation, pp. 44, 56 (2021).
19. Kacal, Merve, "Assessing the impact of modulating protein degradation pathways in cancer". Karolinska Institutet, Ph.D. dissertation, p. 18 (2022).
20. Graham, J. *et al*., *Anal. Biochem*., **220(2)**, 367-373 (1994).
21. Borenfreund, E., and Puerner, J.A., "A simple quantitative procedure using monolayer cultures for cytotoxicity assays (HTD/NR-90)". *J. Tissue Cult. Meth*., **9**, 7-9 (1985).
22. Weeks, J.M., and Svendsen, C., "Neutral red retention by lysosomes from earthworm (*Lumbricus rubellus*) coelomocytes: a simple biomarker of exposure to soil copper". *Environ. Toxicol. Chem*., **15(10)**, 1801-1805 (1996).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

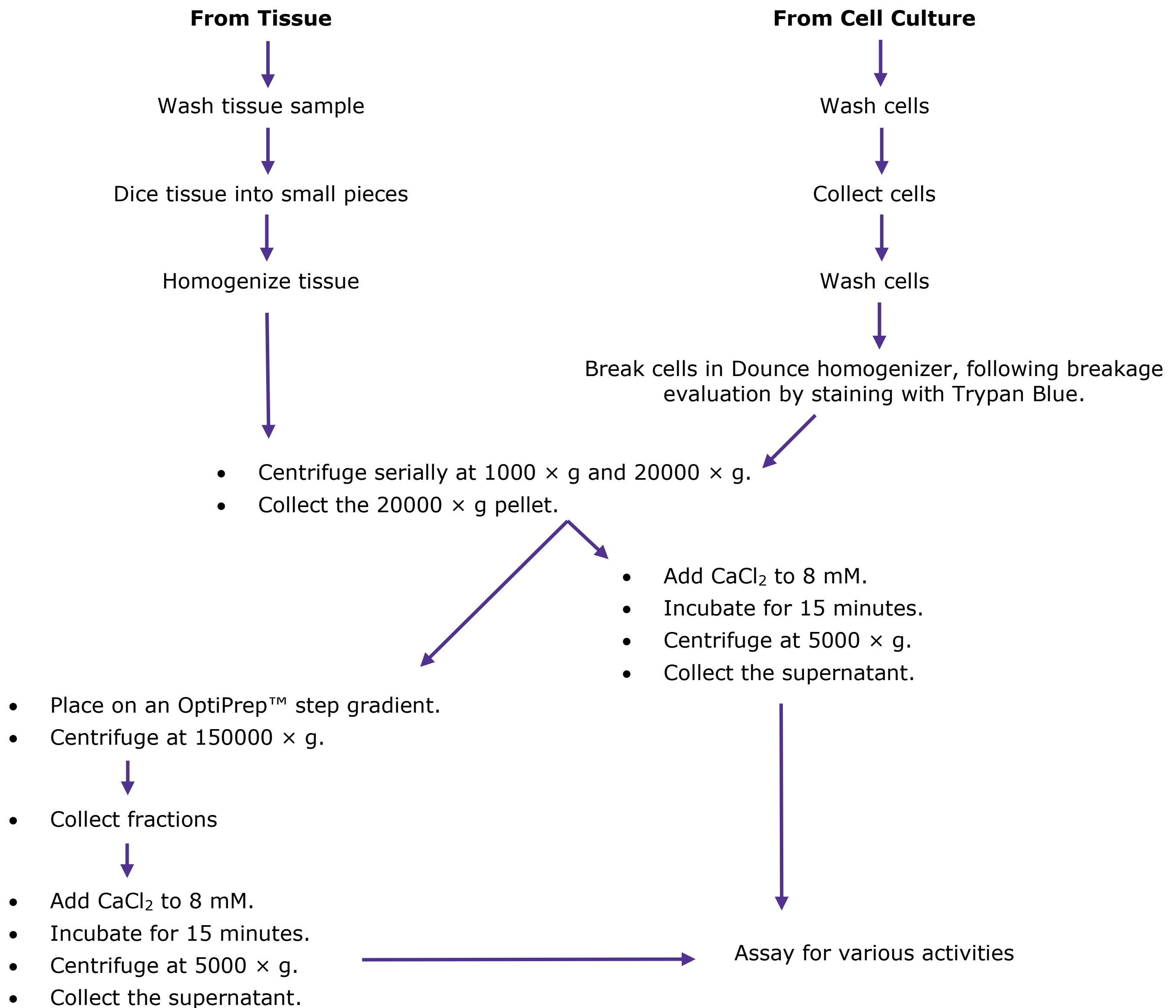
Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices

Appendix I. Flowchart of Lysosome Preparation



The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2007-2023 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

LYSISO1pis Rev 01/23 ESS,PY,GCY,MAM