LeviPrep Mouse Tissue Dissociation Kit

Part Number #1005001
For Research Use Only. Not for use in diagnostic procedures.

Product Description

The LeviPrep™ Mouse Tissue Dissociation Kit has been developed for fast and easy cold dissociation of brain, lung and liver primary tissue. Tissues often require a combination of enzymatic and mechanical force to break structural adhesions or extracellular matrix that hold the tissue intact. In order to release single cells, different enzyme mixtures can be used to target specific tissue and cell types. The LeviPrep Mouse Tissue Dissociation Kit provides enzymes to cover a variety of different tissue dissociations. Protocols are provided for work with mouse brain, lung, and liver tissues. The enzymes in this kit and the protocols included are optimized for highly viable and high yielding dissociated cells for use in downstream studies. Cells can be run on the **LeviCell System** prior to those downstream studies to enrich for viable cells, as needed.

It is often beneficial to work with tissues from perfused mice as it avoids contamination from RBCs. If this is not desirable for your application, see recommendations for RBC removal at the end of this guide.

This kit provides up to 24 digestions of 50-250 mg of tissue. Proper scale-up and calculation of enzyme and buffers are required for larger tissue sample sizes.

Kit Components

Component	Part Number	Quantity	Storage Conditions
Enzyme A	6000030	3x 100 mg	Lyophilized 4°C Resuspended 4°C
Enzyme B	6000031	4x 25 mg	Lyophilized 4°C Resuspended 4°C
Enzyme C	6000032	1x 25 mg	Lyophilized 4°C Resuspended 4°C
Enzyme D	6000033	2x 25 mg	Lyophilized 4°C Resuspended 4°C

The kit provides enzymes in a lyophilized form. Upon arrival, store the kit at 4°C to maintain shelf life. Follow the "Enzyme Preparation" section for reconstitution recommendations.

Additional Tools and Consumables Required:

- BSA
- FBS
- PBS
- P1000 Pipette
- P1000 wide bore tips
- 50 mL conical tube
- 70 µm filter (fits 50 mL conical)
- 0.2 µm sterile filter
- 3 ml syringe
- Ice
- 2.0 mL low binding microcentrifuge tubes
- Razor blade or scalpel
- Cell culture dish for mincing the tissue
- Timer
- Centrifuge with swinging bucket rotor
- Counting method (trypan blue/hemocytometer/ microscope or automated cell counter)
- Analytical balance

Tunable Optimization Parameters

This protocol is intended to be executed at 4°C (on wet ice). If the protocol needs to be optimized for the intended application, there are several parameters that can be tuned to achieve an optimized result.

Enzyme Concentration

Enzyme activity is decreased by reduced temperature, so changing the enzyme concentration used in a tissue dissociation protocol is one way to tune the conditions for an optimal outcome from the digest.

Digestion Temperature

Digestion temperature can be optimized for the application. Maximum enzyme activity is normally achieved at physiological temperatures (i.e., 37°C). However, maximal enzyme activity may not be required to achieve acceptable cell yields and viability metrics for a given application, and there may be benefits to performing the digestion with sub-maximal enzymatic activity. For example, performing the digestion on ice will quiesce cellular activity and responses to the dissociation process.

Digestion Time

This protocol is designed to be completed in 30 minutes, but can be shortened or extended by the user to optimize results.

Enzyme Selection

The LeviPrep Mouse Tissue Dissociation Kit provides multiple enzymes to enable dissociation of different tissues. Not all enzymes are required for all tissues. The enzymes to be used for each specific tissue type are indicated in Table 1.

Tissue	Brain	Liver	Lung
Enzyme A	-	X	X
Enzyme B	X	-	-
Enzyme C	X	X	X
Enzyme D	-	-	X

Table 1. List of enzymes required for each tissue type.

Enzyme Preparation

The enzymes are shipped in a lyophilized form. For longest stability, only remove and prepare enough enzyme needed for your experiments. After resuspension, the enzymes can be aliquoted to smaller volume vials and stored at 4°C. However, this may reduce the enzyme stability, and will have to be evaluated prior to use in your specific application.

Enzyme A: Resuspended at a concentration of 6 mg/mL using 1X PBS. Incubate the resuspended powder at 30°C to completely dissolve the enzyme.

Enzyme B: Resuspended at a concentration of 2 mg/mL using 1X PBS. Incubate the resuspended powder at 37°C to completely dissolve the enzyme. The final suspension should be filtered through a 0.22 µm filter prior to use.

Enzyme C: Resuspended at a concentration of 12,500 Units/mL using 1X PBS.

Enzyme D: Resuspended at a concentration of 430 Units/mL using 1X PBS. Incubate the resuspended powder at 30°C to facilitate resuspension.

**Note that the number of units per mg of lyophilized enzyme may vary depending on the lot. As a result, a different mass of enzyme may be required from one batch to the next.*

Enzyme Mix Preparation

This protocol is designed for use with 50 mg - 250 mg of tissue per reaction, which utilizes 1.9 mL of enzyme. Larger quantities of tissues can be processed, but the enzyme volume will need to be scaled accordingly, and larger tubes selected to accommodate the increased final volume of tissue and enzymatic solution. Prepare enzyme mixes as indicated in Table 2.

Tissue	Brain	Liver	Lung
Enzyme A (6 mg/mL)	-	2 mL	2 mL
Enzyme B (2 mg/mL)	2 mL	-	-
Enzyme C (12,500 U/mL)	20 µL	20 µL	20 µL
Enzyme D (430 U/mL)	-	-	20 µL

Table 2. Enzyme volume needed for dissociation of each tissue type.

Prepare Buffers

For each dissociation, prepare 15 mL of 1X PBS containing 10% FBS. This will be used for quenching the enzymes, and rinsing the dissociated tissue through the filter. In addition, 10 mL of 1X PBS containing 0.5% BSA is needed for the final suspension of cells and wash.

Tissue Dissociation Protocol

1) Mince 50 mg - 250 mg tissue using a scalpel, razor blade, or other appropriate dissecting instrument. Minimal tissue chunks should remain after mincing (2-3 mins max). Prior to mincing the tissue, we recommend recording the tissue mass to gauge the final yield of cells.
2) Transfer minced tissue to a 2 mL microcentrifuge tube.
3) Add 1.9 mL of enzyme mix to the tissue.
4) Triturate 10 times with a wide bore 1 mL pipet tip and place on ice for 30 minutes.
5) Triturate 10 times every 10 minutes for the 30 minute incubation (i.e., at T=0, 10, 20, 30min).
6) Pass digested material through a pre-wetted 70 µm filter into a 50 mL conical tube.
7) Use the plunger from a 3 mL (or similar) syringe to gently push through the tissue material remaining in the filter. Use 10 mL PBS with 10% FBS to help rinse the tissue through the filter. This will also quench the enzymes used in the dissociation.
8) Spin cell suspension for 5 minutes at 300 RCF.
9) Discard the supernatant and keep the pellet.
10) Resuspend the pellet in 10 mL PBS with 0.5% BSA.
11) Spin 5 minutes at 300 RCF.
12) Discard supernatant and keep the pellet.
13) Resuspend pellet in 1 mL PBS with 0.5% BSA or media. This final volume can be adjusted based on the type and size of tissue being dissociated, as well as the desired final concentration of cells.
14) The final suspension of cells can be counted to determine the final yield of cells (normalized to the input mass of tissue if that was recorded), as well as the final viability of the collected cells.

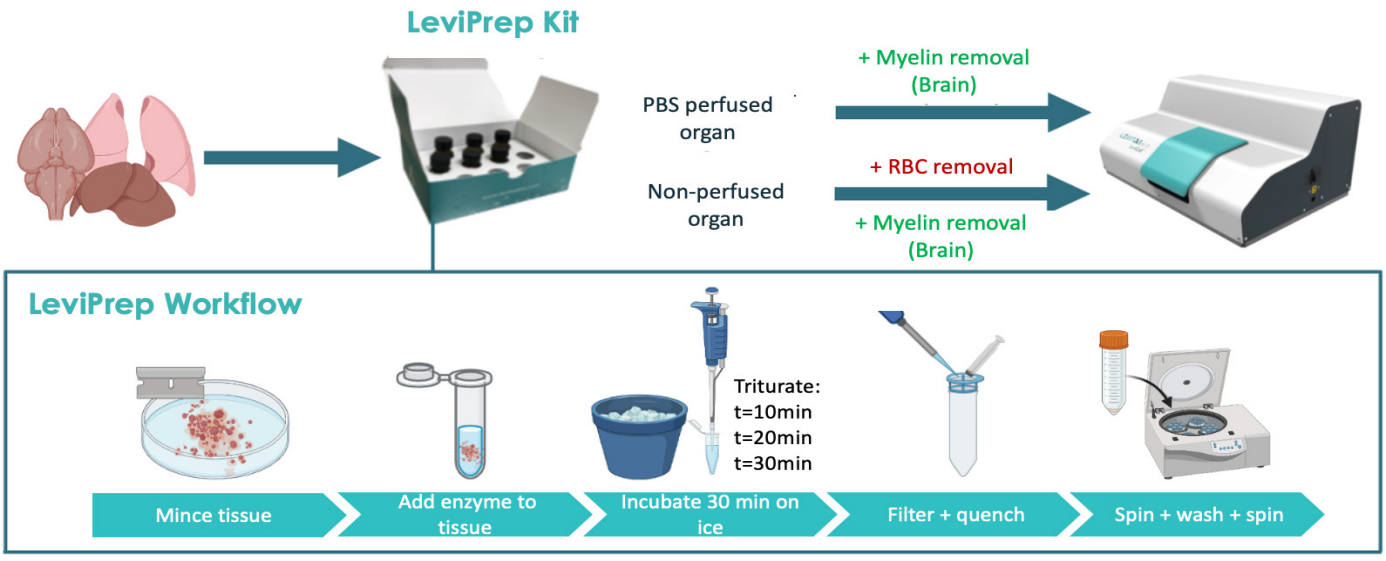

Figure 1. Schematic representation of the LeviPrep tissue dissociaton workflow.

Additional Cleanup Suggestions

LeviCell Viable Cell Enrichment

Quality of tissue digestion can vary due to tissue health and prior handling. Additional processing steps may be required to remove dead cells or debris that may have resulted from the tissue digestion. Enrichment of viable cells can be obtained using the LeviCell System. For further details, refer to documents **LeviCell Instrument User Guide (#90-00204)** and **Quick Reference Guide for Viable Cell Enrichment (#90-00213).**

RBC Removal

If perfusion was not performed, the tissue will likely contain a large number of red blood cells (RBCs) that will contaminate the final cell suspension. There are multiple ways to remove red blood cells from a digested tissue. The most common method is RBC lysis using an isotonic solution. RBC lysis solution is commercially available, but can also be made fresh.

Protocol testing was performed using the manufacturer's recommendations from:
Alfa Aesar by Thermo Fisher Scientific (Fisher Scientific part number AAJ62150AK)

NOTE: *After lysis the sample will contain a large amount of debris. Removal of the final buffer after quenching the lysis reaction and pelleting the cells will remove a significant amount of this debris. At the same time, this step will also remove a number of dead cells resulting in an increase in viability of the cell sample.*

Myelin Removal

Brain tissue frequently contains a large amount of myelin, particularly when working with the adult mouse brain. Myelin is released from brain tissue during digestion and confounds downstream studies including cell counting. If the brain is non-perfused, residual RBCs will also need to be removed. For additional protocols refer to **Adult Mouse Brain Protocols (#90-00073).**

Protocol and testing was performed through modification of a density centrifugation protocol1. The following steps provide a quick and easy way to remove myelin from perfused tissue. Refer to **Protocol "C" in Adult Mouse Brain Protocols (#90-00073).**

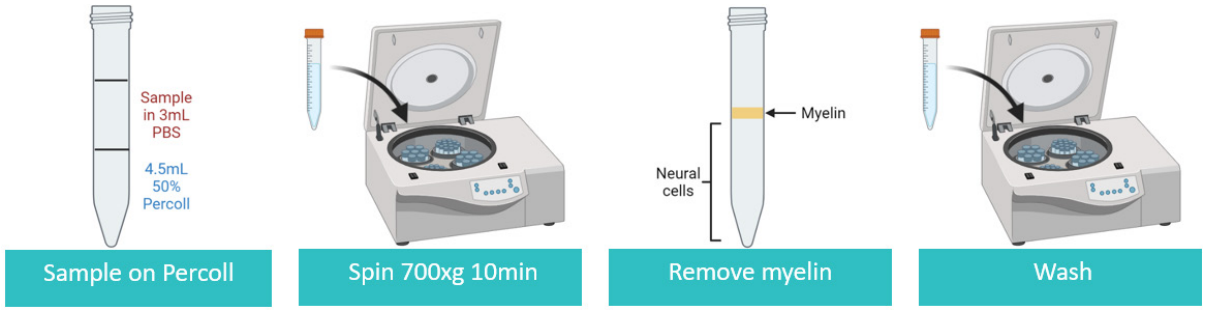

Figure 2. Schematic representation of the myelin removal protocol from an adult perfused brain.

Myelin Removal Protocol

1) Resuspend your cells in 3mL of 1X PBS. Add them on top of 4.5mL of 50% Percoll prepared in a 15mL conical tube.
2) Spin 10 min at 700g, with no brake.
3) Remove myelin ring.
4) Wash residual Percoll and spin 10 min at 700g at 4°C
5) Remove supernatant and resuspend the pellet in 1X PBS with 0.5% BSA or media.

Caution

The following warnings and precautions apply to all enzymes included in this kit.

Warning and Precautions

- Hazard: Irritant/sensitizer.
- Precaution: Do not inhale.
- Precaution: Wear gloves/mask/eye protection.

References

1- Nikodemova, M., Watters, J.J. Efficient isolation of live microglia with preserved phenotypes from adult mouse brain.
J Neuroinflammation 9, 147 (2012). https://doi.org/10.1186/1742-2094-9-147

Acknowledgements

All the schematic representations of protocols were created with BioRender.com.