ALS Mouse Tissue Processing V.1

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IN DEVELOPMENT

ABSTRACT

This protocol describes how to prepare tissues and blood for downstream applications after the ALS mouse study.

PROTOCOL CITATION

katesama 2022. ALS Mouse Tissue Processing. **protocols.io** https://protocols.io/view/als-mouse-tissue-processing-ch9nt95e

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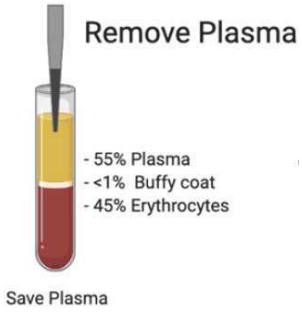
Blood & Plasma

1 Blood and Plasma Processing

1.1 After cardiac puncture and blood collection in K3-EDTA tubes, invert the tube a few times to ensure mixing with EDTA coating and keep it at RT until centrifugation **within 4 hours of collection**.

1.2 Spin **whole blood** at 1,600xg for 10 minutes at RT.

1.3 After centrifugation, pipette the plasma off the top. Be careful not to disturb the clot at the bottom of the tube or the buffy coat, and do not aspirate any of the clot or blood cells. **Place the plasma into a second vial.**



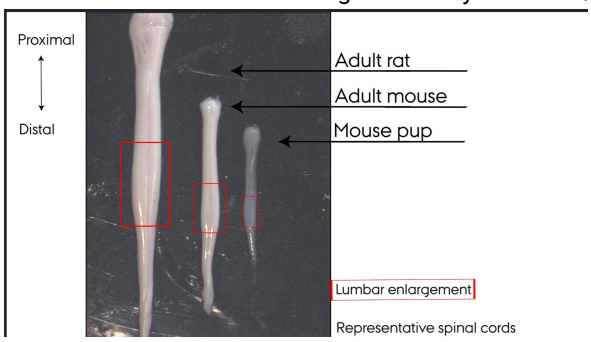
1.4 Plasma can be stored at -20°C for short-term storage (<3 months). Store at -80°C for long-term storage.

Brain

2 Extract and place the **whole brain** in 10mL 10% formalin (approx 1:10 tissue:fixative) and then place the tube on ice.

Spinal Cord

3 Dissect the **lumbar region** of the spinal cord (red box) with a scalpel. Place in 10 mL 10% formalin and put tube on ice ***OR*** in 10 mL 2.5% glutaraldehyde 0.1mol/l cacodylate buffer to fix for electron microscopy at RT.



3.1 Place **the remaining spinal cord sections** in a cryovial and flash-freeze in LN_2.

Gastrocnemius Muscle

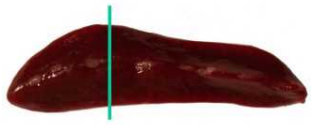
4 Gently stretch both pieces of muscle for 10s by holding onto each side of the tissue with forceps and lightly pulling.

4.1 Place **both pieces** of the gastrocnemius muscle in 10% formalin (approx 1:10 tissue: fixative) and then place the tube on ice ***OR*** in 10 mL 2.5% glutaraldehyde 0.1mol/l cacodylate buffer to fix for electron microscopy and leave at RT *(no need to flash-freeze any muscle).*

Liver, Spleen and Kidneys

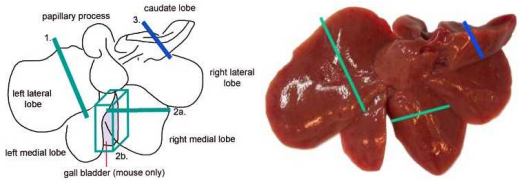
5 Liver, Spleen, and Kidney Processing (for molecular biology and histology)

5.1 Section the spleen by cutting a transverse cross-section at the largest extension of the spleen using a scalpel



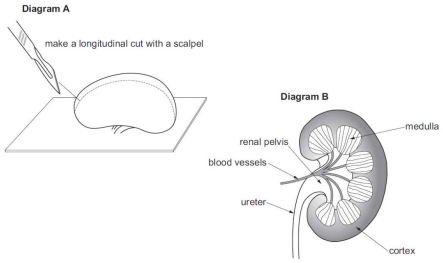
5.2 **First**, use tweezers to place the smaller section in 10 mL 10% formalin (approx 1:10 tissue:fixative) and then place the tube on ice. **Then,** place the second section in a cryovial and flash-freeze in LN_2.

5.3 Section the liver by cutting a transverse cross-section through the left lateral lobe (1) using a scalpel.



5.4 **First**, use tweezers to place the section of the left lateral lobe in 10 mL 10% formalin (approx 1:10 tissue:fixative) and then place the tube on ice. **Then,** place the remaining liver section in a cryovial and flash-freeze in LN_2.

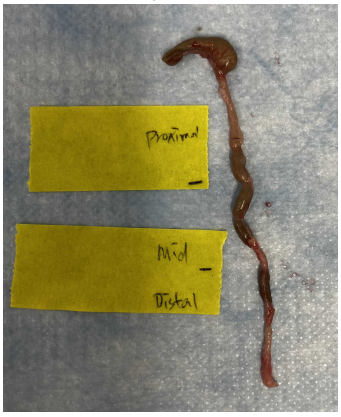
5.5 Section the kidneys by making a longitudinal cut through each kidney using a scalpel.



5.6 **First**, use tweezers to place a section of each kidney in one tube of 10 mL 10% formalin (approx 1:10 tissue: fixative) and then place the tube on ice. **Then,** place the remaining sections in a cryovial and flash-freeze in LN_2.

Colon

6 **First**, lay out the colon flat as pictured and discard the proximal section. **Then**, separate the mid and distal sections using scissors or a scalpel.



6.1 Using the back of a pair of forceps, gently push on the colon to remove the contents.

6.2 Cut the **mid-section** in half and place **one half** in 1mL RNAlater solution. Then, adjust the **second half** of the mid-section into a tube shape using closed forceps and place it in 1mL of 10% formalin. Store both tubes on ice.

6.3 Place the **distal** section in a cryovial and flash-freeze in LN_2.

Fixation and Cryprotection

7 Fix formalin samples overnight at 4C. Then, replace 10% formalin with cold 30% sucrose in PBS. Leave overnight at 4C, then embed or store in PBS at 4C until embedding.

7.1 Leave tissue overnight at 4C in sucrose solution, then embed or store in PBS at 4C until embedding.

RNA Sample Storage

8 Leave colon samples in RNAlater overnight at 4C to allow the solution to penetrate the tissue. Then, flash-freeze the samples in LN_2 and transfer them to -80.

Cryovial Storage

9 After returning to the lab and spinning down blood, transfer all samples in the -80 and log the samples, their position, and details of the sacrifice in the spreadsheet.