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REFERENCES FOR IMAGING

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PROTOCOLS

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FIXED BRAIN SECTIONING
PROTOCOL FOR CUTTING SLICES OF FIXED BRAIN TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Deeply anesthetize animal, then transcardially perfuse with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4.
    - Note that you can perfuse the animal in a variety of ways, including gravity-feed, pump, or push-syringe.
2. Remove brains by dissection and fix in 4% phosphate buffered paraformaldehyde at 4° C overnight.
    - Be careful not to fix your tissue for too long before cutting and processing it! Over-fixation will decrease good protein staining when you do immunohistochemistry. So don't forget to take out the fixed brain after 24 hours.
3. Pour out the paraformaldehyde and then cryoprotect the fixed brain with 30% sucrose buffer at 4° C overnight.
4. Pour out the 30% sucrose solution. If you are not sectioning the brain right away, store it in PBS until you are ready for cutting. If you are ready to section with the Compresstome®, rinse the brain in PBS first.
5. Select a section of the brain that you would like to take cut for slices.
6. Glue the tissue sample onto the Compresstome® specimen syringe.
7. Draw the syringe downward to bring the brain tissue core sample into the syringe.
8. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    - Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
9. Cool the entire contents of the specimen syringe with the chilling block. The brain tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
10. Load the specimen syringe onto the Compresstome® slicer.
11. The protocol is complete for preparing the fixed brain specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

- What are the optimal settings on the Compresstome® for cutting fixed brain slices? Try a speed (Advance) of 2 and an oscillation of 4-6. We have found that these parameters work best for obtaining superb brain slices with smooth surfaces without chattermarks.

References

* Uses the Compresstome® for successful fixed brain slices.

1. Abdelaal HM, Kim HO, Wagstaff R, Sawahata R, Southern PJ, Skinner PJ. Comparison of Vibratome and Compresstome sectioning of fresh primate lymphoid and genital tissues for in situ MHC-tetramer and immunofluorescence staining. Biol Proced Online. 2015 Jan 7;17(1):2. doi: 10.1186/s12575-014-0012-4.

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PRECISIONARY

FRESH BRAIN SECTIONING
PROTOCOL FOR CUTTING SLICES OF FRESH BRAIN TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Deeply anesthetize animal.
2. Remove brains by dissection. If you are not sectioning the brain right away, store it in PBS until you are ready for cutting.
3. If you are ready to section with the Compresstome®, rinse the brain in PBS first.
4. Select a section of the brain that you would like to take cut for slices.
5. Glue the tissue sample onto the Compresstome® specimen syringe.
6. Draw the syringe downward to bring the brain tissue core sample into the syringe.
7. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    - Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
8. Cool the entire contents of the specimen syringe with the chilling block. The brain tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
9. Load the specimen syringe onto the Compresstome® slicer.
10. The protocol is complete for preparing the fixed brain specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

- What are the optimal settings on the Compresstome® for cutting fresh brain slices? Try a speed (Advance) of 2 and an oscillation of 4-6. We have found that these parameters work best for obtaining superb brain slices with smooth surfaces without chattermarks.

References

* Uses the Compresstome® for successful fresh brain slices.

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PRECISIONARY

EYE TISSUE SECTIONING
PROTOCOL FOR CUTTING SLICES OF EYE TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks
-

1. Obtain eye tissue from animal of choice.
2. Immediately freeze in OCT and/or formalin fixed and embedded in paraffin.
3. Select a section of the eye tissue that you would like to take cut for slices.
4. Glue the tissue sample onto the Compresstome® specimen syringe.
5. Draw the syringe downward to bring the eye tissue core sample into the syringe.
6. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    a. Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
7. Cool the entire contents of the specimen syringe with the chilling block. The eye tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
8. Load the specimen syringe onto the Compresstome® slicer.
9. The protocol is complete for preparing the lymph node for sectioning. Proceed from here with normal Compresstome® sectioning procedures

References

* Uses the Compresstome® for successful eye tissue slices.

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FROZEN TISSUE SECTIONING
PROTOCOL FOR CUTTING FROZEN TISSUE WITH THE COMPRESSTOME®

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Take frozen tissue samples and thaw them in either ice-cold 10% formalin or 4% paraformaldehyde. Make formalin and paraformaldehyde using 1X PBS solution.

√ Thawing in fixation will help preserve the integrity and architecture of the tissue specimen.

- Do NOT thaw tissue specimens directly at room temperature. This will dry out your tissue sample.

2. Thaw tissue samples for at least 24 hours. Afterwards, samples should be stored at 4 °C.

3. Once tissue is thawed and fixed, continue with agarose embedding of the sample to use with the Compresstome® vibratome for sectioning tissue slices.

- Try a cutting speed of 1.5 and an oscillation of 3 for cutting fixed tissue samples, and then adjust accordingly.

PRECISIONARY

HEART TISSUE SECTIONING
PROTOCOL FOR CUTTING SLICES OF HEART TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Deeply anesthetize animal with pentobarbital, 70 mg/kg.
    - Note that you can perfuse the animal in a variety of ways, including gravity-feed, pump, or push-syringe.
2. Remove hearts by dissection and perfuse in Langendorff-mode with bicarbonate-buffered solution.
    - Note that fat accumulation at the epicardial surface increases with animal age. Fat tissue is difficult to cut and can blunt the blade, so we recommend careful manual removal of fat tissue before slicing.
3. Load dye via the coronary circulation, apply by injecting into the aortic cannula. Use 22 µl of a solution containing the voltage-sensitive dye di-4-ANBDQPQ and then Pluronic F-127 (2 µl of a 20% stock solution in DMSO. Add over a 4 to 5 minute time period.
4. After dye loading, perfuse hearts at room temperature with BDM-containing HEPES-buffered solution.
5. Select a section of the heart that you would like to take cut for slices.
6. Glue the tissue sample onto the Compresstome® specimen syringe.
7. Draw the syringe downward to bring the brain tissue core sample into the syringe.
8. Fill the syringe with 4% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    - Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
9. Cool the entire contents of the specimen syringe with the chilling block. The heart tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
10. Load the specimen syringe onto the Compresstome® slicer.
11. The protocol is complete for preparing the heart specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

References

* Uses the Compresstome® for successful heart tissue slices.

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PRECISIONARY

IMMUNOHISTOCHEMISTRY
BASIC PROTOCOL FOR PROTEIN STAINING IN FIXED TISSUE SLICES

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Obtain cut slices using normal Compresstome® sectioning procedures.
   √ You can store free-floating sections in PBS or other buffer solutions for about 3-4 weeks at 4° C. Select out the slices you want to stain for IHC, and store the rest until you do more experiments.

2. For immunohistochemistry, first rinse free-floating sections three times in phosphate buffered saline (PBS), 5 min each time.

3. Incubate sections in 10% normal goat serum for non-specific blocking, 1 hour.

4. Incubate sections in primary antibody at 4° C overnight (24 hours).

5. Rinse free-floating sections three times in phosphate buffered saline (PBS), 5 min each time.

6. Include sections in secondary antibody at 25° C (room temperature), 1 hour.

7. Rinse free-floating sections three times in phosphate buffered saline (PBS), 5 min each time.

8. Using a paintbrush, mount sections onto glass slides, then air dry sections.

9. Coverslip slides with mounting media, such as Vectashield mounting media (Vector Laboratories).
   √ For fluorescent IHC, be sure to cover your slides or keep them in the dark. Do not expose the sections to sunlight, or the protein signal will decrease.

References

* Uses the Compresstome® for for successful immunohistochemistry staining.

1. Abdelaal HM, Kim HO, Wagstaff R, Sawahata R, Southern PJ, Skinner PJ. Comparison of Vibratome and Compresstome sectioning of fresh primate lymphoid and genital tissues for in situ MHC-tetramer and immunofluorescence staining. Biol Proced Online. 2015 Jan 7;17(1):2.

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IN-SITU HYBRIDIZATION
PROTOCOL FOR IN-SITU HYBRIDIZATION (DNA & RNA)

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. For in-situ hybridization (ISH), fresh tissue should be fixed in 4% paraformaldehyde overnight, then rinsed in 1X PBS the next day. Tissue should be sectioned with the Compresstome® for desired slices.
    √ Be sure to not "over-fix" tissue in paraformaldehyde! Doing so results in excessive cross-linking of proteins that can interfere with getting good signals for in-situ hybridization.
- What slice thickness should you aim for? For ISH, slices should be 3 µm to 7 µm thick. They can be as thick as 10 µm.

2. Mount free-floating slices onto Superfrost-Plus slides (Fisher Scientific).
    √ Why use Superfrost-PLUS slides? Because there is a (+) charge on these types of slides and allow tissue slices to adhere to the slide surface. Therefore, when you are processing the slides, the slices won't just fall off.

3. Dry slices on slide using a slide warmer for 30 min at 37°C.

4. Fix slides again in 4% paraformaldehy in diethyl pyrocarbonate (DEPC) H2O for 20 min.

5. Rinse slides with DEPC PBS (2 x 5 min).

6. Deproteinate slices with proteinase K treatment (50 µg.ml; 1M Tris HCl at pH 7.5, and 0.1M EDTA) for 30 min at 25°C.

7. After deproteination, rinse sections in DEPC PBS (2 x 5 min).

8. Fixed slides in 4% paraformaldehyde in DEPC H2O for 20 min.

9. Briefly rinse slides in DEPC H2O.

10. Next, hybridize slices in hybridization buffer containing the oligonucleotides you are using. Incubate for 24 hours at 37°C in a humid chamber.

11. After hybridization, wash sections in 0.2X SSC (4 X 20 min each) at 47°C.

12. Block slices in blocking protein in Tris buffered saline (TBS) for 30 min (Perkin Elmer Amplification Kit).

13. Incubate slides in digitoxigenin-horseradish peroxidase in TBS containing Tween 20 (TBST; 8 µl/ml) for 30 min at 25°C.

14. Rinse slides in TBST (3 X 5 min).

15. Incubate slides in tyramide solution (1:50 in kit diluent) for 10 min at 25°C.

16. inse slides in TBST (3 X 5 min).

17. Incubate slides in streptavidin-F (1:50) in TNB buffer (0.05 g/ml block protein in Tris-saline) for 30 min.

18. Rinse slides in TBST (3 X 5 min).

19. Air dry slides and mount coverslips using Vectashield Mounting Media.

References

* Uses the Compresstome® for successful in-situ hybridization.

1. Abdelaal HM, Kim HO, Wagstaff R, Sawahata R, Southern PJ, Skinner PJ. Comparison of Vibratome and Compresstome sectioning of fresh primate lymphoid and genital tissues for in situ MHC-tetramer and immunofluorescence staining. Biol Proced Online. 2015 Jan 7;17(1):2.

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PRECISIONARY

PRECISION-CUT GUT SLICES
PROTOCOL FOR CUTTING SLICES OF FRESH GUT TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Prepare Krebs-Henseleit Buffer (KHB) for slicing intestine and storing intestinal slices.
    - Here's the simple recipe for KHB:

KHB (slicing buffer)	Concentration (mM)
NaCl	118
KCl	5
MgSO4·7H2O	1.1
KH2PO4	1.2
NaHCO3	25
CaCl2·2H2O	2.5
D-Glucose	25
HEPES	9

2. Prepare Williams medium E (WME, containing L-glutamine)
    - Here's the simple recipe for KHB:

WME	Concentration (mM)
WME + L-glutamine	5
D-Glucose	14
Gentamycin	50 µg/ml
Amphotericin B	2.5 µg/ml

- Amphotericin B is added to inhibit growth of fungi and yeasts.

3. Fill the culture plates you will use with the incubation medium.
4. Warm and oxygenate culture plates with 95% O2/5% CO2 at 37 C for at least 30 min.
5. Prepare and keep warm a 2-3% solution of agarose at 37 C.

6. To obtain intestinal tissue, anesthetize the animal and retrieve the gut as quickly as possible, ideally under 5 minutes. Remove any fatty tissue and flush the intestines by pipetting ice-cold oxygenated KHB solution. Transfer cleaned pieces of intestinal tissue to ice-cold oxygenated KHB.

- Intestinal tissue is very sensitive to ischemia, so the time taken for dissection and incubation of precision-cut gut tissue should be as fast as possible.

7. Tighten one end of the gut segment with surgical thread. Using a transfer pipette, fill the intestine with 2-3% agarose. Close the end of intestine with thread or a clamp, then transfer into cold oxygenated KHB to help solidify the agarose into a gel (<1 min required).
8. Once agarose has solidified, cut the intestinal segment into sections that you want to slice.
9. Glue the tissue sample onto the Compresstome® specimen syringe.
10. Draw the syringe downward to bring the intestinal tissue core sample into the syringe.
11. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).

- Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit!

12. Cool the entire contents of the specimen syringe with the chilling block. The intestinal tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
13. Load the specimen syringe onto the Compresstome® slicer.
14. The protocol is complete for preparing the intestinal specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

- What are the optimal settings on the Compresstome® for cutting live intestinal slices? Try a speed (Advance) of 4 and an oscillation of 5-7.

References

* Uses the Compresstome® for successful eye tissue slices.

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PRECISIONARY

PRECISION-CUT KIDNEY SLICES
PROTOCOL FOR CUTTING SLICES OF FRESH KIDNEY TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Prepare Krebs-Henseleit Buffer (KHB) for slicing and storing precision-cut kidney slices.
   - Here's the simple recipe for KHB:

KHB (slicing buffer)	Concentration (mM)
NaCl	118
KCl	5
MgSO4·7H2O	1.1
KH2PO4	1.2
NaHCO3	25
CaCl2·2H2O	2.5
D-Glucose	25
HEPES	9

- Adjust the pH of the solution to 7.42 slowly by adding drops of 5N NaOH solution.
- The KHM solution can be stored for 24 hours at 4°C.

2. Prepare Williams medium E (WME, containing L-glutamine)
   - Here's the simple recipe for KHB:

WME	Concentration (mM)
WME + L-glutamine	5
D-Glucose	14
Gentamycin	50 µg/ml
Amphotericin B	2.5 µg/ml

- Amphotericin B is added to inhibit growth of fungi and yeasts.
- The WME solution can be stored for 24 hours at 4°C.

3. Fill the culture plates you will use with the incubation medium (WME incubation medium)
4. Warm and oxygenate culture plates with 95% O2/5% CO2 at 37 C for at least 30 min.
5. Prepare and keep warm a 2-3% solution of agarose at 37 °C.

6. To obtain kidney tissue, anesthetize the animal and surgically isolate the kidney as quickly as possible, ideally under 5 minutes. Remove any fatty tissue and transfer cleaned pieces of kidney tissue to ice-cold oxygenated KHB.
    - Kidney tissue is very sensitive to ischemia, so the time taken for dissection and incubation of precision-cut kidney slices should be as fast as possible. This is especially important for studies of kidney metabolism.
7. Glue the selected kidney sample onto the Compresstome® specimen syringe plunger. Place the embedding cap onto the specimen syringe, over the kidney sample.
8. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
9. Draw the syringe downward to bring the kidney tissue core sample into the syringe.
10. Cool the entire contents of the specimen syringe with the chilling block. The kidney tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
11. Load the specimen syringe onto the Compresstome® slicer.
12. The protocol is complete for preparing the kidney specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures. Use KHB slicing solution (see above) for the buffer tray.
    - What are the optimal settings on the Compresstome® for cutting live kidney slices? Try a speed (Advance) of 2-3 and an oscillation of 5-7.

√ The biggest challenge of slicing precision-cut kidney slices is getting consistent sections when there is hard (fibrotic) kidney tissue. The Compresstome® has patented compression technology that allows for smooth, even slices and overcomes this obstacle.

13. Collect each kidney slice and immediately transfer to pre-warmed Williams' Medium E (see recipe above), incubating at 37° C to restore ATP levels and wash away cellular debris.

References

* Uses the Compresstome® for successful kidney slices.

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PRECISION-CUT LIVER SLICES
PROTOCOL FOR CUTTING SLICES OF FRESH LIVER

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Prepare Krebs-Henseleit Buffer (KHB) for slicing and storing precision-cut liver slices.
   - Here's the simple recipe for KHB:

KHB (slicing buffer)	Concentration (mM)
NaCl	118
KCl	5
MgSO4·7H2O	1.1
KH2PO4	1.2
NaHCO3	25
CaCl2·2H2O	2.5
D-Glucose	25
HEPES	9

2. Prepare Williams medium E (WME, containing L-glutamine)
   - Here's the simple recipe for KHB:

WME	Concentration (mM)
WME + L-glutamine	5
D-Glucose	14
Gentamycin	50 µg/ml
Amphotericin B	2.5 µg/ml

- Amphotericin B is added to inhibit growth of fungi and yeasts.

3. Fill the culture plates you will use with the incubation medium.
4. Warm and oxygenate culture plates with 95% O2/5% CO2 at 37 C for at least 30 min.
5. Prepare and keep warm a 2-3% solution of agarose at 37 C.

6. To obtain liver tissue, anesthetize the animal and surgically isolate the liver as quickly as possible, ideally under 5 minutes. Remove any fatty tissue and transfer cleaned pieces of liver tissue to ice-cold oxygenated KHB. Section out specific liver lobes you need for experiments.
    - Liver tissue is very sensitive to ischemia, so the time taken for dissection and incubation of precision-cut liver tissue should be as fast as possible. This is especially important for studies of liver metabolism.
7. Glue the selected liver sample onto the Compresstome® specimen syringe plunger. Place the embedding cap onto the specimen syringe, over the liver sample.
8. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    - Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
9. Draw the syringe downward to bring the liver tissue core sample into the syringe.
10. Cool the entire contents of the specimen syringe with the chilling block. The liver tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
11. Load the specimen syringe onto the Compresstome® slicer.
12. The protocol is complete for preparing the liver specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.
    - What are the optimal settings on the Compresstome® for cutting live liver slices? Try a speed (Advance) of 4-5 and an oscillation of 5-7.
13. Collect each liver slice and immediately transfer to pre-warmed Williams' Medium E (see recipe above), incubating at 37° C to restore ATP levels and wash away cellular debris.

References

* Uses the Compresstome® for successful fresh liver slices.

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PRECISIONARY

PRECISION-CUT LUNG SLICING
PROTOCOL FOR CUTTING SLICES OF FRESH LUNG TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Anesthetize the animal, then dissect to cannulate the exposed trachea with a catheter.
2. Cool the live lung specimen with 4°C saline.
3. (Optional) Inject a bolus of agarose gel (2%) to fill the pulmonary artery.
    - This process of filling with agarose allow for inflation of alveoli with the same embedding material, and prevents the alveoli from collapsing during the cutting process. Some lung specimens may be sectioned without agarose infusion, especially if the lung specimens are from bovine or sheep animal models (because the quantity of agarose needed for infusion exceeds several liters).
4. Using a syringe, inject a bolus of air to clear the airway.
    - Injection of air helps infused agarose reach the alveoli.
5. Select a section of the lung lobe you would like to take tissues from for sections.
6. Glue the tissue sample onto the Compresstome® specimen syringe. Place the embedding cap onto the tube, then fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C). Tap the side of the tube to dispel any bubbles from the agarose.
    - Loading the agarose with the embedding cap allows you to see if there are any bubbles surrounding the specimen *before* you draw the tube plunger down.
7. Draw the syringe downward to bring the lung tissue core sample into the syringe.
8. Cool the entire contents of the specimen syringe with the chilling block. The lung tissue sample is now embedded in agarose. The agarose will solidify enough for stable sectioning.
9. Load the specimen syringe onto the Compresstome® slicer.
10. The protocol is complete for preparing the lung tissue core specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

- PCLSs produced can be maintained overnight in Dulbecco's modified Eagle medium (37°C, 5% CO2) supplemented with 1% penicillin-streptomycin solution.

References

* Uses the Compresstome® for successful lung tissue slices.

1. *Graham JG, Winchell CG, Kurten RC, Voth DE. Development of an Ex Vivo Tissue Platform To Study the Human Lung Response to Coxiella burnetii. Infect Immun. 2016 Apr 22;84(5):1438-45.
2. *Hiorns JE, Bidan CM, Jensen OE, Gosens R, Kistemaker LE, Fredberg JJ, Butler JP, Krishnan R, Brook BS. Airway and Parenchymal Strains during Bronchoconstriction in the Precision Cut Lung Slice. Front Physiol. 2016 Jul 21;7:309.
3. *Oenema TA, Maarsingh H, Smit M, Groothuis GM, Meurs H, Gosens R. Bronchoconstriction Induces TGF-β Release and Airway Remodelling in Guinea Pig Lung Slices. PLoS One. 2013 Jun 26;8(6):e65580.
4. *Royce SG, Nold MF, Bui C, Donovan C, Lam M, Lamanna E, Rudloff I, Bourke JE, Nold-Petry CA. Airway Remodeling and Hyperreactivity in a Model of Bronchopulmonary Dysplasia and Their Modulation by IL-1 Receptor Antagonist. Am J Respir Cell Mol Biol. 2016 Dec;55(6):858-868.
5. *Yim PD, Gallos G, Perez-Zoghbi JF, Trice J, Zhang Y, Siviski M, Sonett J, Emala CW Sr. Chloride channel blockers promote relaxation of TEA-induced contraction in airway smooth muscle. J Smooth Muscle Res. 2013;49:112-24.

PRECISIONARY

LYMPH NODE TISSUE SECTIONING
PROTOCOL FOR CUTTING SLICES OF LYMPH NODE TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Obtain lymph node and spleen from animal of choice.
2. Immediately freeze in OCT and/or formalin fixed and embedded in paraffin.
3. Select a section of the spleen that you would like to take cut for slices.
4. Glue the tissue sample onto the Compresstome® specimen syringe.
5. Draw the syringe downward to bring the lymph node tissue core sample into the syringe.
6. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    1. Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
7. Cool the entire contents of the specimen syringe with the chilling block. The lymph node tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
8. Load the specimen syringe onto the Compresstome® slicer.
9. The protocol is complete for preparing the lymph node for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

References

* Uses the Compresstome® for successful lymph node tissue slices.

1. Li S, Folkvord JM, Rakasz EG, Abdelaal HM, Wagstaff RK, Kovacs KJ, Kim HO,Sawahata R, MaWhinney S, Masopust D, Connick E, Skinner PJ. Simian Immunodeficiency Virus-Producing Cells in Follicles Are Partially Suppressed by CD8+ Cells In Vivo. J Virol. 2016 Nov 28;90(24):11168-11180. Print 2016 Dec 15. PubMed PMID: 27707919; PubMed Central PMCID: PMC5126374.

2. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, Kaisho T, Bogunovic D, Bhardwaj N, Krummel MF. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. Cancer Cell. 2016 Aug 8;30(2):324-36. doi:10.1016/j.ccell.2016.06.003. Epub 2016 Jul 14. PubMed PMID: 27424807; PubMed Central PMCID: PMC5374862.

PRECISIONARY

MUSCLE TISSUE SECTIONING
PROTOCOL FOR CUTTING SLICES OF MUSCLE TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Extract the specific muscle tissue out you want to use your research.
2. Select a section of the muscle that you would like to take cut for slices.
3. Glue the tissue sample onto the Compresstome® specimen syringe.
4. Draw the syringe downward to bring the muscle tissue core sample into the syringe.
5. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    a. Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
6. Cool the entire contents of the specimen syringe with the chilling block. The muscle tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
7. Load the specimen syringe onto the Compresstome® slicer.
8. The protocol is complete for preparing the muscle tissue specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

References

* Uses the Compresstome® for successful muscle tissue slices.

1. Yim PD, Gallos G, Perez-Zoghbi JF, Trice J, Zhang Y, et al. Chloride channel blockers promote relaxation of TEA-induced contraction in airway smooth muscle. J. Smooth Muscle Res. 2014 Mar 21; 49: 112–124. doi: 10.1540/jsmr.49.112.

2. Gallos G, Yocum GT, Siviski ME, Yim PD, Xiao WF, et al. Selective targeting of the α5-subunit of GABAA receptors relaxes airway smooth muscle and inhibits cellular calcium handling. American Journal of Physiology Online. 2015 May 1(309)9: L931-L942. DOI: 10.1152/ajplung.00107.2014.

3. Danielsson J, Perez-Zoghbi JF, Bernstein K, Barajas MB, Zhang Y, et al. Antagonists of the TMEM16A Calcium-activated Chloride Channel Modulate Airway Smooth Muscle Tone and Intracellular Calcium. Anesthesiology Online. 2015 Sept. 1;(123) 569-581. doi:10.1097/ALN.0000000000000769.

4. Castro-Piedras I, Perez-Zoghbi JF. Hydrogen sulphide inhibits Ca2+ release through InsP3 receptors and relaxes airway smooth muscle J Physiol Online. 2013; 59(23) pp 5999–6015. DOI: 10.1113/jphysiol.2013.257790.

PRECISIONARY

PLANT SECTIONING PROTOCOL
COMPRESSTOMETM FROM PRECISIONARY INSTRUMENTS

1. Complete procedure for using the CompresstomeTM slicer for sectioning plant materials
2. Examples of plant sections cut at varying thicknesses (5 µm – 100 µm)
3. Examples of plant leaf sections
4. Examples of plant seed sections
5. Additional protocols for treatment of sections
6. Additional protocols for visualizing plant vasculature in sections

Sectioning Slices of Plant Tissue

This document summarizes the basic laboratory techniques needed for sectioning plant materials (both fixed and fresh specimens) with the CompresstomeTM slicer. The CompresstomeTM VF-200, VF-200-0Z, VF-300, and VF-300-0Z models are all compatible with sectioning plant tissues. Solid plant materials can be sectioned in several planes for researchers to explore the various tissues within plant leaves, stems, roots, and seeds. Creating slices with the CompresstomeTM is an excellent alternative to paraffin methods.
Please note that the VF-200 and VF-200-0Z have a precision setting of 10 µm, whereas the VF-300 and VF-300-0Z have precision of 1 µm. Therefore, it's recommended to use the VF-300 if sectioning slices < 50 µm.

Protocol for Plant Sectioning

Embedding and Sectioning of Plant Materials:

1. Select the plant material (leaf, flower, bud, stem, root, or seed) that you would like to section. As an example, below is an image of a newly sprouted sweet pea seed that will be sectioned (**Figure 1A**).
2. Glue the plant specimen to the top of the CompresstomeTM specimen tube, and let the plant briefly dry to adhere to the tube top (**Figure 1B**).

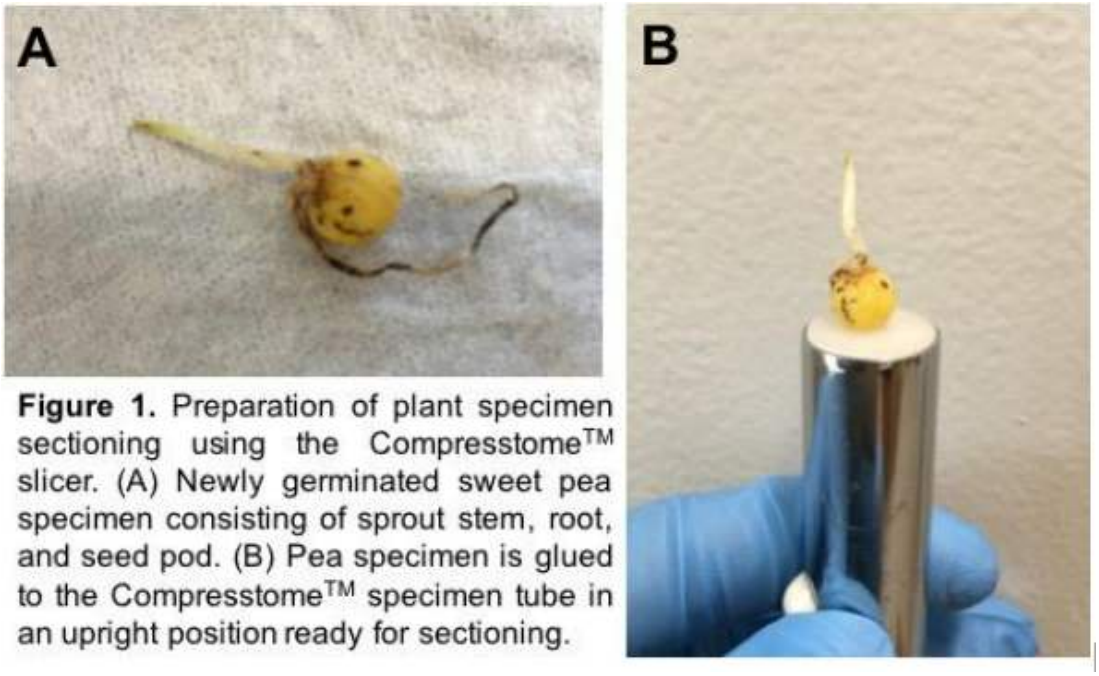


Figure 1. Preparation of plant specimen sectioning using the Compresstome™ slicer. (A) Newly germinated sweet pea specimen consisting of sprout stem, root, and seed pod. (B) Pea specimen is glued to the Compresstome™ specimen tube in an upright position ready for sectioning.

3. Place the agarose loading cap onto the specimen tube, making sure that it is level across the top of the tube.
4. Pipette agarose into the loading cap so that the entire plant specimen is covered in agarose. Gently tap the sides of the tub and loading cap to dispel any air bubbles from inside the agarose.
5. Gently draw down the specimen tube so that the plant specimen enters the metal tube casing along with the agarose.
6. Please the tube containing the specimen in ice, or chill it in the refrigerator for 5 min.
7. Remove the tube from the chiller and remove the loading cap. The embedded specimen in agarose is now ready to be sectioned.
8. Please follow the instructions manual for your CompresstomeTM for sectioning your desired thickness of slices.

Treatment of Sections:

Sections of fresh plant material after cutting should be placed in water as floating sections before mounting onto glass slides or for other experimental use. Preserved and treated plant materials after cutting should be immersed into alcohol. Some experimental techniques will require long rinses or dehydration methods for plant materials. In these cases, sections may be moved to different dishes or incubators with a small watercolor brush.

Mounting of Slices Containing Plant Materials:

After sections are completed, you can stain or treat the plant slices as desired for your experimental needs. To mount the slices, place sections into water or alcohol so that they are free-floating. Using a small watercolor brush, gently sweep the section onto a glass slide (preferred slides are Fisherbrand Superfrost Plus Glass Slides). Wipe any excess liquid off with a tissue and let the slide air dry. Once dried, place 2-3 drops of Vectashield Mounting Media (from Vectashield) onto a glass coverslip, and then coverslip the top of the mounted specimen. Use nail polish to paint the edges of the coverslip to help hold the glass in place and seal in the mounting media so that your specimen can be preserved for imaging (**Figure 2**).

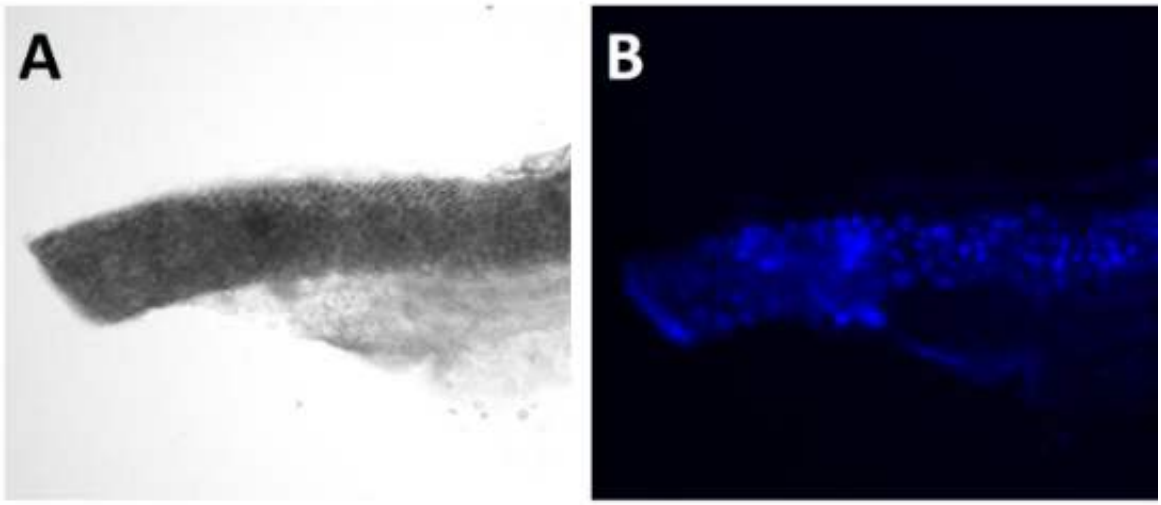


Figure 2. Examples of sectioned plant leaves. Sections that were 50 µm thick were taken from a sprouted sweet pea plant. (A) Transection slice of the leaf shown in bright field microscopy. Note that individual plant cells are distinguishable. (B) Same section shown through a fluorescent blue/cyan filter for DAPI staining of plant cell nuclei.

Best CompresstomeTM Settings for High Quality Plant Slices

The CompresstomeTM VF-300 is capable of sectioning plant materials from 5 µm to 100 µm thick. Examples of plant leaf slices are shown in Figure 2 above, and slices of varying thicknesses are shown in Figure 3 below. To achieve the best plant slice, try using these troubleshooting techniques:

1. Set the oscillation to a range of 1-2. A lower oscillation setting for slicing helps prevent tearing of plant tissue during the cutting process. Whereas a higher oscillation is generally preferred for mammalian tissues, plant tissues are much more prone to tearing at higher oscillation settings.

ge of 7-8. A high speed setting for slicing plants helps make a clean cut through the plant material. This is very different compared to mammalian tissue sectioning, because lower speeds are generally preferred for mammalian specimens. However, plants require a more rapid cutting motion to prevent tearing. This is especially true for sectioning germinating seeds, because a slow cutting speed causes the seed material to disintegrate during slicing. A faster speed helps preserve the seed tissue and allows it to remain intact and embedded in the agarose as an entire section (See **Figure 4** for examples of seed sections that have preserved ultrastructure made using the CompresstomeTM).

3. When attempting to slice very thin sections (<30 µm thickness), it is best to begin sectioning at 50 µm to 100 µm- thick slices, then gradually decrease the thickness down to the desired thickness. The gradual decrease in sectioning thickness helps you make consistent slices as you begin to create very thin slices that are much more delicate to maneuver (See examples in **Figure 3**).

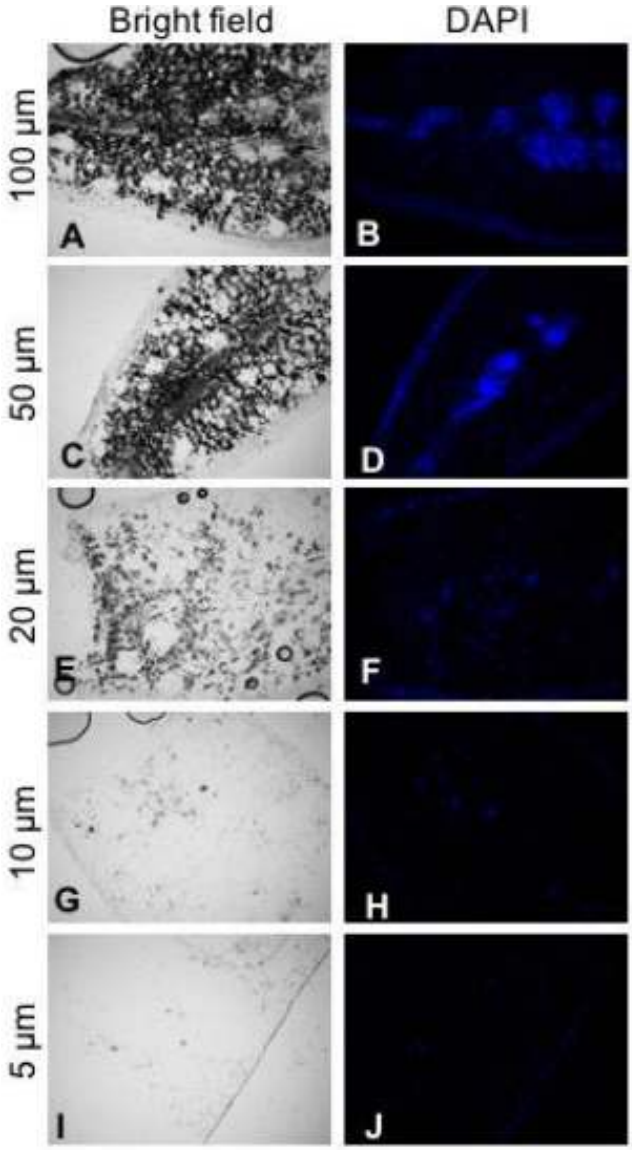


Figure 3. Sections of plant leaf at various thicknesses cut using a Compresstome VF-300. A sweet pea plant leaf was embedded in agarose and sectioned to yield slices that are 100 µm (A-B), 50 µm (C-D), 20 µm (E-F), 10 µm (G-H), and 5 µm (I-J) thick. Images in the left column were taken with a bright field microscope; images in the right column are corresponding sections taken with blue flouroscence to depict DAPI staining.

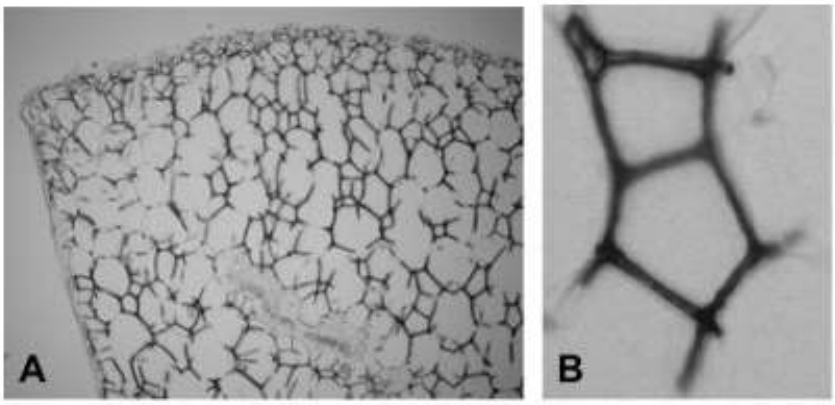


Figure 4. Sections of plant seed made with a Compresstome VF-300 slicer. (A) Depicts a 50 µm-thick section cut from a sweet pea plant seed. Image taken at 40X with a bright field microscope, showing preservation of the cell wall ultrastructure. (B) Magnification of the plant seed section, showing the preservation of the honeycomb-pattern of plant cell walls that comprise the seed.

Visualization of Intact Vascular Systems of Leaves and Flowers

Some experiments require the ability to clearly visualize the vascular systems of leafy plants and floral parts. The simplest method for achieving clarity of plant specimens while preserving the intact vascular systems is with the following technique, which aims to remove hydrophobic pigments such as chlorophyll:

1. Clear plant specimens in 5% NaOH in a Petri dish in an oven set to 37 °C. The time needed for clearing will vary from one to several days depending on the texture and composition of the plant material.
2. Remove plant specimens from the oven, and rinse 3-5 times in distilled water carefully with a pipette.
3. If additional clearing is needed, place the plant specimens in a saturate aqueous solution of chloral hydrate for 24 hours. Then wash 3-5 times in distilled water.

PRECISIONARY

SKIN TISSUE SECTIONING
PROTOCOL FOR CUTTING SLICES OF SKIN TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Extract the specific skin tissue out you want to use your research.
2. Select a section of the skin that you would like to take cut for slices.
3. Glue the tissue sample onto the Compresstome® specimen syringe.
4. Draw the syringe downward to bring the skin tissue core sample into the syringe.
5. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    a. Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
6. Cool the entire contents of the specimen syringe with the chilling block. The skin tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
7. Load the specimen syringe onto the Compresstome® slicer.
8. The protocol is complete for preparing the skin tissue specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

References

* Uses the Compresstome® for successful skin tissue slices.

1. Nia HT, Liu H, Seano G, Datta M, Jones D, Rahbari N, et al. Solid stress and elastic energy as measures of tumour mechanopathology. Nat. Biomed. Eng. 1, 0004. 2016 Nov 28; 18;(1):1. doi:10.1038/s41551-016-0004.

PRECISIONARY

SPINAL CORD SECTIONING
PROTOCOL FOR CUTTING SLICES OF SPINAL CORD

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

This protocol can be adapted for either obtaining live slices of spinal cord (for electrophysiology and imaging) or fixed slices of spinal cord (for immunohistochemistry or in-situ hybridization).

1. For live spinal cord slices: First deeply anesthetize animal, then transcardially perfuse with saline to clear out blood in all organ systems. Dissect out the spinal cord and rapidly cool it in pre-chilled cutting solution.
2. For fixed spinal cord slices: First deeply anesthetize animal, then transcardially perfuse with saline to clear out blood in all organ systems, followed by 4% paraformaldehyde infused for 10 minutes. Dissect out the spinal cord and store it in 4% paraformaldehyde overnight, or for at least 24 hours before sectioning.
    - Be careful not to fix your tissue for too long before cutting and processing it! Over-fixation will decrease good protein staining when you do immunohistochemistry. So don't forget to take out the fixed spinal cord after 24 hours. Rinse the tissue in PBS and store it in 1X PBS until you are ready to cut slices with the Compresstome®.

Sectioning spinal cord slices with the Compresstome®:

3. Select a section of the spinal cord that you would like to take cut for slices.
4. Cut out a rectangular shape of solidified agarose or gelatin that is slightly larger than the spinal cord specimen itself.
5. Place (don't glue!) the spinal cord specimen on top of the rectangular piece of agarose/gelatin, and then glue one end on to the Compresstome® specimen syringe.
    The rectangular piece of agarose/gelatin helps hold the spinal cord as it is embedded into the agarose and specimen syringe.
6. Draw the syringe downward to bring the spinal cord core sample into the syringe.
7. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    - Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
8. Cool the entire contents of the specimen syringe with the chilling block. The spinal cord is now embedded in agarose. The agarose will solidify enough for stable sectioning.

9. Load the specimen syringe onto the Compresstome® slicer.
10. The protocol is complete for preparing the spinal cord specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

- What are the optimal settings on the Compresstome® for cutting spinal slices? For fixed tissue, try a speed (Advance) of 2 and an oscillation of 4-6. For live tissue, try a speed of 4 and an oscillation of 5-7. We have found that these parameters work best for obtaining superb spinal cord slices with smooth surfaces without chattermarks.

References

* Uses the Compresstome® for successful spinal cord slices.

1. Abdelaal HM, Kim HO, Wagstaff R, Sawahata R, Southern PJ, Skinner PJ. Comparison of Vibratome and Compresstome sectioning of fresh primate lymphoid and genital tissues for in situ MHC-tetramer and immunofluorescence staining. Biol Proced Online. 2015 Jan 7;17(1):2. doi: 10.1186/s12575-014-0012-4.

2. Duncan J, Kersigo J, Gray B, Fritzsch B. Combining lipophilic dye, in situ hybridization, immunohistochemistry, and histology. J Vis Exp. 2011 Mar 17;(49).

3. Selever J, Kong JQ, Arenkiel BR. A rapid approach to high-resolution fluorescence imaging in semi-thick brain slices. J Vis Exp. 2011 Jul 26;(53).

4. Ting JT, Daigle TL, Chen Q, Feng G. Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. Methods Mol Biol. 2014;1183:221-42.

HOW TO EMBED SPINAL CORD FOR THE COMPRESSTOME® TISSUE SLICER

1 Make agarose: usually ~2% concentration

2 Make the agarose base for holding the spinal cord

(This step helps stabilize the spinal cord for embedding in the Compresstome® specimen tube)

2a. Pour half of the agarose solution into a small petri dish

2b. Store the remaining half of the agarose solution in a hot water bath (~37°C) so that it does not solidify

2c. Solidify the agarose in the petri dish at 4°C

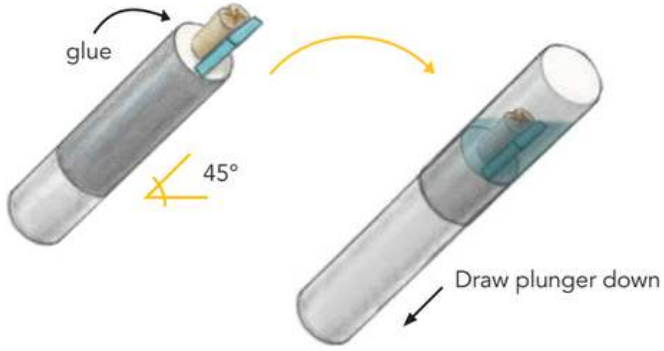
2d. After the agarose solidifies, cut out a small rectangular block of agarose about 5 mm wide x 3 mm tall x 3 mm long.

3 Dissect out the spinal cord tissue that you would like to section with the Compresstome® Vibratome

3a. Lay the spinal cord longitudinally onto the small agarose block

3b. Cut off one end so that both the spinal cord and agarose block are even

4 Embed the spinal cord in the agarose in the specimen tube



4a. Glue the spinal cord and agarose block together to the surface of the white plunger. (Tip: keep everything at a 45° angle when loading the spinal cord and agarose block)

4b. Slowly draw down the white plunger until the entire specimen is inside the tube.

4c. Using a transfer pipette, add agarose solution to cover the entire content of the specimen tube

4d. Chill with chilling block

4e. Now you're ready to slice spinal cord tissue!

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(617) 682-0586
www.precisionary.com
info@precisionary.com

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SPLEEN TISSUE SECTIONING
PROTOCOL FOR CUTTING SLICES OF SPLEEN TISSUE

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Obtain spleen from animal of choice.
2. Immediately freeze in OCT and/or formalin fixed and embedded in paraffin.
3. Select a section of the spleen that you would like to take cut for slices.
4. Glue the tissue sample onto the Compresstome® specimen syringe.
5. Draw the syringe downward to bring the spleen tissue core sample into the syringe.
6. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
    a. Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !
7. Cool the entire contents of the specimen syringe with the chilling block. The spleen tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
8. Load the specimen syringe onto the Compresstome® slicer.
9. The protocol is complete for preparing the spleen tissue for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

References

* Uses the Compresstome® for successful spleen tissue slices.

1. Li S, Folkvord JM, Rakasz EG, Abdelaal HM, Wagstaff RK, Kovacs KJ, Kim HO,Sawahata R, MaWhinney S, Masopust D, Connick E, Skinner PJ. Simian Immunodeficiency Virus-Producing Cells in Follicles Are Partially Suppressed by CD8+ Cells In Vivo. J Virol. 2016 Nov 28;90(24):11168-11180. Print 2016 Dec 15. PubMed PMID: 27707919; PubMed Central PMCID: PMC5126374.

1. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, Kaisho T, Bogunovic D, Bhardwaj N, Krummel MF. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. Cancer Cell. 2016 Aug 8;30(2):324-36. doi:10.1016/j.ccell.2016.06.003. Epub 2016 Jul 14. PubMed PMID: 27424807; PubMed Central PMCID: PMC5374862.

PRECISIONARY

CUTTING TUMOR SLICES
PROTOCOL FOR SECTIONING LIVE & FIXED TUMOR SPECIMENS

Key to reading the protocol:

√ Rationale for procedural step

- Tips & Tricks

1. Make 2% agarose using low-gelation temperature agarose (Sigma-Aldrich) or using the Precisionary agarose tablets. Mix with PBS buffer to dissolve.
2. Dissect and excise the tumor, and wash with PBS.
3. Glue the tumor specimen to the Compresstome® specimen tube, then embed with 2% agarose solution. Cool immediately with the pre-chilled chilling block to solidify the agarose gel.

- Pre-chill the chilling block for 10 min in the freezer or in ice water.

4. Load the specimen tube containing the tumor tissue onto the Compresstome® vibratome and begin cutting using normal procedures. The agarose that surrounds the tumor will help hold it in place and allow the tumor to be sectioned with minimal displacement.
5. For fixed tumor slices: place tumor slices in 4% paraformaldehyde for at least 24 hours, then rinse with PBS before further experimental processing.
6. For live tumor slices, immerse tumor slices in PBS for at least 10 min, then incubate per your own experimental protocols.

- What are the optimal settings on the Compresstome® for cutting tumor slices? Try an oscillation of 3-4 and an advance (speed) of 2. We find that these parameters yield the best tumor sections.

References

* Uses the Compresstome® for successful tumor slices (live and fixed).

10. Askoxylakis V et al. Preclinical Efficacy of Ado-trastuzumab Emtansine in the Brain Microenvironment. J Natl Cancer Inst. 2015Nov 7;108(2).

2. Boldajipour B et al. Tumor-infiltrating lymphocytes are dynamically desensitized to antigen but are maintained by homeostatic cytokine. J CI Insight. 2016 Dec 8;1(20):e89289.

3. Broz ML et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. **Cancer Cell**. 2014 Nov10;26(5):638-52.

4. Nia H et al. Solid stress and elastic energy as measures of tumour mechanopathology. **Nature Biomedical Engineering** 2016; 1:0004.