



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
MgSO ₄ ·7H ₂ O	1.1
KH ₂ PO ₄	1.2
NaHCO ₃	25
CaCl ₂ ·2H ₂ O	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
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- √ 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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2. Lerche-Langrand C, Toutain HJ. Precision-cut liver slices: characteristics and use for in vitro pharmaco-toxicology. *Toxicology.* 2000 Nov 16;153(1-3):221-53.

3. Koch A, Saran S, Tran DD, Klebba-Färber S, Thiesler H, Sewald K, Schindler S, Braun A, Klopfleisch R, Tamura T. Murine precision-cut liver slices (PCLS): a new tool for studying tumor microenvironments and cell signaling *ex vivo*. *Cell Commun Signal*. 2014 Nov 7;12:73.
4. Abdelaal HM, Kim HO, Wagstaff R, Sawahata R, Southern PJ, Skinner PJ. Comparison of Vibratome and Compressome 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.