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
$MgSO_4 \cdot 7H_2O$	1.1
KH_2PO_4	1.2
$NaHCO_3$	25
$CaCl_2 \cdot 2H_2O$	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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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.

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