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
$MgSO_4 \cdot 7H_2O$	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 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.**

1. **Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. Cell. 2016 Jun 16;165(7):1762-75.**

2. de Graaf IA, Olinga P, de Jager MH, Merema MT, de Kanter R, van de Kerkhof EG, Groothuis GM. Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. Nat Protoc. 2010 Sep;5(9):1540-51.

3. de Kanter R, Tuin A, van de Kerkhof E, Martignoni M, Draaisma AL, de Jager MH, de Graaf IA, Meijer DK, Groothuis GM. A new technique for preparing precision-cut slices from small intestine and colon for drug biotransformation studies. J Pharmacol Toxicol Methods. 2005 Jan-Feb;51(1):65-72.

4. de Kanter R, Monshouwer M, Meijer DK, Groothuis GM. Precision-cut organ slices as a tool to study toxicity and metabolism of xenobiotics with special reference to non-hepatic tissues. Curr Drug Metab. 2002 Feb;3(1):39-59.