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
$MgSO_4 \cdot 7H_2O$	1.1
KH_2PO_4	1.2
$NaHCO_3$	25
$CaCl_2 \cdot 2H_2O$	2.5
D-Glucose	25
HEPES	9

   - √ 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 WME:

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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2. Poosti F, Pham BT, Oosterhuis D, Poelstra K, van Goor H, Olinga P, Hillebrands JL. Precision-cut kidney slices (PCKS) to study development of renal fibrosis and efficacy of drug targeting ex vivo. Dis Model Mech. 2015 Oct 1;8(10):1227-36.

3. Stribos EG, Hillebrands JL, Olinga P, Mutsaers HA. Renal fibrosis in precision-cut kidney slices. Eur J Pharmacol. 2016 Nov 5;790:57-61.

4. Vickers AE, Fisher RL. Precision-cut organ slices to investigate target organ injury. Expert Opin Drug Metab Toxicol. 2005 Dec;1(4):687-99.