Isolation of Epithelial Cells from Mouse Gastrointestinal Tract for Western Blot or RNA Analysis

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[Abstract] The gastrointestinal (GI) tract is lined by a single layer of epithelial cells which function in secretion, absorption, and digestion. In addition, most GI tract tumors develop from epithelial cells (carcinomas). This protocol describes isolation of the surface epithelium from the underlying stroma, muscular layer and submucosa in the GI tract. In this protocol, epithelial cell adhesions are weekend by chelating Ca +2 ions followed by mechanical separation of the cells by vortexing. Analysis of protein levels and gene expression patterns in isolated epithelial cells versus whole GI tissue minimizes the potential for confounding contributions from contaminating stromal cells.

Materials and Reagents

1. 4% sodium hypochlorite (Sigma-Aldrich, catalog number: 239305)
2. Phosphate buffered saline (PBS) (Mediatech, Cellgro®, catalog number: 21-031)
3. KCl
4. NaCl
5. KH_2PO_4
6. Na_2HPO_4
7. EDTA
8. DTT
9. Aprotinin (USB, catalog number: 9087-70-1)
10. Leupeptin (MP, catalog number: 195623)
11. Pepstatin (MP, catalog number: 195368)
12. PMSF (Sigma-Aldrich, catalog number: 329-98-5)
13. Ethanol
14. 0.04% sodium hypochlorite (see Recipes)
15. 100x protease inhibitor (store at -20 °C) (see Recipes)
16. Solution B (see Recipes)
17. 1x lysis buffer (see Recipes)

Equipment

1. CO_2 chamber for mice
2. Dissecting scissors (one medium size and one small size for dissecting mice)
3. Forceps (2)
4. 15-ml conical tubes
5. 1.5-ml Eppendorf tubes
6. 3 ml Insulin syringe
7. Vortex
8. Centrifuge compatible with 15-ml conical

Procedure

1. Make sure to prepare all the solutions including PBS at least 1 h before the start of killing the mice and store them on ice so they are cold when you start to work with them.
2. Sacrifice the mouse (18-25 grams) by CO_2 asphyxiation according to American Veterinary Medical Association (AVMA) guidelines on Euthanasia, 2007 (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf). Briefly, first carbon dioxide's anesthetic properties are used by exposing rodents to a slowly rising concentration of carbon dioxide until they become unconscious. A chamber fill rate of 20%, or 1/5 th of the cage volume per minute is recommended. Slower, uncoordinated movements occur after about 1 min, animals stop moving after about 2 min, and unconsciousness occurs after about 2.5 min. After unconsciousness is achieved, flow can be increased to hasten death. Two-step euthanasia is used to ensure death. Once the animal has lost consciousness and is unresponsive to a toe pinch, a follow-up method is utilized. This can include decapitation with sharp scissors, cervical dislocation, or thoracotomy.
3. Open the abdomen and dissect the gastrointestinal tract from the stomach to anus by pulling gently on the stomach and removal of the mesentry (the peritoneal folding around the intestine which connects the intestinal tract to the dorsal abdominal wall. It contains fat, blood vessels and lymph nodes).
4. Cut the intestine into pieces of interest (Jejunum, Ileum and large intestine).
5. Keep the pieces in cold PBS on ice and work with them one at a time.
6. Flush the content of the intestine with cold PBS to a 3 m clean it using l insulin syringe. Alternatively, invert the intestine on a glass rod and swirl in cold PBS
7. Wash intestinal pieces in cold PBS and keep them until you are done with all pieces (to synchronize your work).

8. Incubate pieces in 50- 100 ml of 0.04% sodium hypochlorite on ice for 15 min (this works well using a small beaker). This step removes bacterial contaminants.
9. During this time prepare three 15-ml conical tubes for every tissue piece and put 5-10 ml of solution B in each tube (if you are using glass rods. 10 ml will be necessary). Mark the tubes and leave them on ice.
10. Remove intestinal pieces from the sodium hypochlorite and rinse in PBS.
11. Put the intestinal pieces in the 15 ml conical containing solution B for 15 min on ice.
12. Remove solution B and add 5 ml PBS (or solution B). I do this by holding the intestinal piece with a long forceps, decanting the solution B then adding back the intestinal piece and add PBS.
13. Vortex for 15 sec.
14. Take the intestinal pieces and put them in another 15 ml conical with solution B.
15. Repeat steps 11-14 twice (you will have 3 tubes per piece).
16. Suspend the cells from all tubes for every piece and mix them in one tube.
17. Centrifuge at 1,000 rpm (1,000 *x g*, 10 min at 4 °C).
18. Take the fluid off and suspend the cells in lysis buffer (5 ml for Jejunum and Ileum or 1 ml for colon) for total cell lysate or Trizol (Invitrogen) 1 ml for RNA extraction.

Recipes

1. 0.04% sodium hypochlorite
   1 ml 4% sodium hypochlorite
   99 ml PBS
2. Solution B
   2.7 mM KCl 0.2 g or 2.7 ml 1 M KCl
   150 mM NaCl 8.77 g 50 ml 3 M NaCl solution
   1.2 mM KH_2PO_4 0.16 g
   680 mM Na_2HPO_4 9.65 g
   1.5 mM EDTA 0.44 g 3 ml 0.5 M EDTA solution
   0.5 mM DTT 0.08 g 1 ml 0.5M DTT (add fresh)
   Bring to 1 L in ddH_2O.
3. 100x protease inhibitor (store at -20 °C)
   10 mg aprotinin
   10 mg leupeptin
   10 mg pepstatin
   174.2 mg PMSF

Bring to 10 ml in absolute ethanol

Vortex well before using

4. 1x lysis buffer

5x reporter lysis buffer	2 ml
ddH_2O	7.9 ml
100x protease inhibitor	100 µl

Acknowledgments

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References

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