

Isolation of Colonic Crypts

Adapted from Samuel et al., Am J Physiol Cell Physiol 296: C296-C305, 2009

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Materials

Colon tissue

HBSS

Blade (scalpel, scissors, razor, etc)

1.5mL tubes

Petri dishes

EDTA disodium salt (ethylenediaminetetraacetate)

DTT (dithiotreitol)

PBS

37C water bath

Recipes

0.1 M EDTA = 3.8 g of EDTA.2Na.2H₂O in 100mL dH₂O

10 mM DTT = 0.1542 g of DTT in 100mL PBS

1 mM EDTA with 0.05 mM DTT = .2mL of .1M EDTA + .1mL of 10mM DTT + 19.7mL PBS

1. Harvest colon tissue, cut open and rinse thoroughly with HBSS (Ca and Mg free) to remove all fecal material. This can be done on bibulous paper, a polystyrene dish, or in a conical tube.
2. Cut colons into approximately 1 cm² pieces.
3. Place all colon pieces from same animal in 1.5mL tube with 0.5mL of 1mM EDTA + 0.05mM DTT.
4. Incubate sample in 37C water bath for 30 min.
5. Gently shake the sample to release crypts by inverting the tube ~10 - 20 times.
6. Remove the colon pieces into another 1.5 mL tube with fresh 0.5mL of 1mM EDTA with 0.05mM DTT. Save the suspension of crypts in a small dish.
7. Repeat steps 4-6 two more times, pooling crypt suspensions from each sample.