

Resource Summary Report

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UC Davis - Ex vivo assessment of barrier function-gut permeability

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Type: Protocol

Proper Citation

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.yipfudn>

Authors: Amy Ehrlich, Trina Knotts

Group: Mouse Metabolic Phenotyping Centers

Summary: Gut tissue (by region- e.g ileum & colon) will be opened along the mesenteric border and mounted in Ussing chambers (Physiologic Instruments, San Diego, CA, USA), exposing 0.3 cm² of tissue surface area to 2.5ml of oxygenated Krebs-glucose (10mM) and Krebs-mannitol (10mM) at 37°C on the serosal and luminal sides, respectively. The paracellular pathway and transcellular pathway will be measured as the flux of FITC-Dextran 4000 (FD-4, Sigma –Aldrich) and horseradish peroxidase (HRP Type VI, Sigma Aldrich), respectively. FD-4 (400µg/ml) and HRP (200µg/ml) will be added to the mucosal chamber and samples will be collected from the serosal chamber every 30 min for 2 hours. Concentration of FD-4 is measured via fluorescence at excitation 485 nm, emission 538 nm. O-dianisidine substrate is used to detect HRP at absorbance 450 nm.

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External URL: <https://mmpc.org/shared/document.aspx?id=300&docType=Protocol>

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