INTESTINAL PERMEABILITY ASSAY – FITC-DEXTRAN

Created By: Jessica Breznik
Date: October 17, 2016
v2 Last Modified: January 1, 2018
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

BACKGROUND

This assay is an indirect measure of total intestinal permeability. Mice are given an oral gavage of a fluorescently-labelled small molecule (FITC-dextran) in the assay, and at a subsequent timepoint the difference in fluorescence pre- and post-gavage is measured in plasma.

This assay works best with two or more people – one person does blood collection and the other weighs the microfuge tubes and processes the blood after collection

MATERIALS AND REAGENTS REQUIRED

- Heparinized capillary tubes
- FITC-dextran (3-5 kDa; Sigma-Aldrich FD4)
- Acid-citrate-dextrose solution (Sigma-Aldrich C3821)
- Razor blades (or other sharp blades)
- Sterile 1x PBS
- 1.5mL microfuge tubes (optional: 0.5 mL microfuge tubes)
- Autoclaved gavage needle (one per mouse group)
- 1 mL syringe
- Opaque black flat-bottom 96-well plate
- Timer and Pen

PROCEDURE

Fast mice for 4-6 hours prior to the test, preferably at the beginning of the light cycle (12 hour cycle; 7 am at McMaster CAF) to minimize discomfort to the mice. When fasting, transfer mice to a new cage (to limit coprophagy) without food or bedding, but keep the water bottle in the cage.

Weigh pre-labelled 1.5mL tubes (2 for each sample – before and after gavage) and record their weights. Prepare 150 µL/mouse of 80 mg/mL FITC-dextran in sterile 1x PBS. Note that the syringe will retain part of the volume so it is necessary to prepare extra solution. (Historical note – Netusha previously used a 200 µL/mouse gavage).

After fasting, nick the tail of the mouse about ¾ from the proximal region, wipe with gauze to remove the first drop, and collect one capillary tube of blood (about 50 µL) in a pre-weighed 1.5 mL tube. Place on ice. Repeat for all mice.

Gavage the mouse with 150 µl of 80 mg/ml FITC dextran (4kDa). Start the timer after gavaging the first mouse, and note the time of the last gavage. Blood collection will be repeated at 4 hours post-gavage (this time was determined to be optimal by the Schertzer lab).

For the pre-test blood, weigh the 1.5mL microfuge tubes containing blood and correct for the pre-determined tube weight to determine the weight of the blood.

Add 15% v/v acid-citrate-dextrose solution (i.e. 15% of weight of blood) as an anticoagulant (38 mM citric acid, 107 mM sodium citrate, 136 mM dextrose). Mix thoroughly by inversion. Centrifuge at 5,000 rpm for 10 minutes, and then transfer the plasma to a new 1.5 mL microfuge tube (or 0.5 mL microfuge tube). Keep tubes in dark on ice or transfer to 4˚C refrigerator.

N.B. Some protocols for this assay directly measure fluorescence intensity in whole blood diluted in 1x PBS, but the standard seems to be plasma.

After 4 hours repeat blood collection and processing as for pre-test blood samples to collect plasma.

Dilute the pre- and post-test plasma samples between 1:4 and 1:10 in 1x PBS and transfer to a black opaque-bottom 96-well plate. A total volume of 100uL – usually 10/90 of plasma/PBS – will cover the bottom of the plate. More plasma can be loaded if the signal is too low, and similarly the plasma can be diluted if the signal is too high. Include a PBS blank. Readings of relative fluorescence units by a spectrophotometer may be measured in duplicate or triplicate and averaged. The plate may be covered and stored at 4˚C in the dark prior to the spectrophotometer reading.

Fluorescence is determined at 530 nm with excitation at 485 nm.

Permeability can be expressed as relative fluorescence units between the groups being compared. To determine relative fluorescence units, subtract the PBS blank fluorescence from all samples and then subtract the post-gavage fluorescence from the pre-gavage fluorescence.

N.B. Protocols in the literature do not always include a pre-test fluorescence measurement or account for fluorescence of the diluent solution (i.e. PBS in this protocol), but it is strongly encouraged to do so to ensure that any baseline differences in plasma fluorescence do not mask significant changes post-gavage.