UC Davis MMPC-Live Protocol
In vivo Intestinal Permeability

Version: 1.0
Revision Date: 10/20/2023
Replaces version: None
Edited by: Michael Goodson - UC Davis Metabolism & Metabolic Health Core

Summary:

In the health gastrointestinal tract, 4 kDa dextrans should be unable to cross the intestinal epithelian, therefore, the amount of FITC-conjugated 4kDa dextrant (FD4) detected in the bloodstream after gavage is a measure of the extent of intestinal barrier impairment.

Reagents and Materials:

Reagent/Material	*Vendor*	*Stock Number*
Fluorescein isothiocyanate–4 kDa dextran (FD4)	Sigma	FD4
Goldenrod 5mm lancet	MEDIpoint	
Microvette® 200 EDTA blood collection tube, or equivalent	Sarstedt	20.1288.100
Microcentrifuge (refrigerated)		
Microplate reader capable of reading FITC fluorescence		
Mouse gavage needle(s)		

Protocol:

1. Fast mice to be tested into clean cages with no food (but access to water). Weigh all mice at the time of fasting. Label cage as fasting with date and start/stop times.
2. After 6 hrs of fasting, gavage mice with FITC-conjugated 4 kDa dextran (FD4; Sigma #FD4-1G) at 600 mg/kg body wt (10 µl/g BW of a 60 mg/ml solution).
3. After 1 h, collect at least 120 µl blood via submandibular blood collection using a Goldenrod 5mm Lancet (Medipoint #GR-5MM) into a plasma collection tube (Microvette 200 K3-EDTA, 200 µl Sarstedt #20.1288.100). Staunch bleeding by applying pressure with a 2x2 gauze pad if necessary. Cap and invert tube twice to mix anticoagulant. Place tube on ice while collecting the remaining samples.
4. Return diet to mice.

5. Centrifuge blood at 4°C, 2,000xg, for 10 min. Collect plasma into a fresh microfuge tube on ice.
6. In the meantime, prepare FD4 Standards indicated below. Standards 1-7 and the blank are used for the assay.
7. Add 50 µl of untreated serum or plasma (*e.g.* rat plasma or fetal bovine serum) to all the wells in columns 1 and 2 of a black walled 96 well plate. These are the wells for the standards and blank replicates. Add 50 µl of each standard or blank to duplicate wells.
8. Add 1x PBS to sufficient wells for each mouse sample. Add 50 µl plasma sample wells. If there is insufficient plasma to use 50 µl, then add a smaller (known volume) and make up the difference with untreated serum or plasma. Record the volume and calculate the dilution factor to correct the concentration from that measured by interpolating from the standard curve.
9. Measure FD4 using a microplate fluorescence spectrophotometer (*e.g.* Molecular Devices M2) at the excitation wavelength of 485 nm and the emission wavelength of 535 nm. Calculate the concentration of FD4 in the mouse plasma from the values of the FD4 standards fit with a log/log curve or a 5PLC standard curve

FD4 Standards

Standard	*Diluent*	*Standard #*	*[FD4]*
45 µl of FD4 (60 mg/ml)	*555 µl of PBS*	*A*	*4.5 mg/ml*
50 µl of #A	*450 µl of PBS*	*B*	*0.45 mg/ml*
50 µl of #B	450 µl of PBS	1	45000 ng/ml
150 µl of #1	300 µl of PBS	2	15000 ng/ml
150 µl of #2	300 µl of PBS	3	5000 ng/ml
150 µl of #3	300 µl of PBS	4	1667 ng/ml
150 µl of #4	300 µl of PBS	5	556 ng/ml
150 µl of #5	300 µl of PBS	6	185 ng/ml
150 µl of #6	300 µl of PBS	7	62 ng/ml
--	300 µl of PBS	Blank	0 ng/ml

Based on: Cani PD, Rodrigo B, Knauf C, *et al.* Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008; **57**:1470–1481.

Reagent Preparation:

FD4 Solution: 60 mg/ml FD4 in water suitable for gavage into mice.