Intestinal Permeability (in vivo)

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Ref: Koster ST et al. STAR Protocols 2021

Summary:

This protocol is for the indirect measurement of in vivo intestinal permeability by measuring the appearance of a fluorophore in the blood following oral administration. The intestinal distribution of the fluorophore (FITC) can also be used to measure intestinal transit time.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Fluorescein isothicyanate-dextran (FITC)	Millipore-Sigma	46944 (4000 MW)
Phosphate Buffered Saline (PBS)		
15 ml Eppendorf tubes		
1.5 ml cryovial tubes		
1ml pipette with tips		
Feeding syringes (1 ml)		
Flushing syringes (5 ml)		
Gavage needles		
BD-Monovette lithium heparin tubes	Fisher	02-675-187
Scissors, forceps		
Microplate-SpectraMax Ready	Molecular Device	ID3
Lab coats/gloves/PPE		

Protocol:

1. SET-UP

a. Prepare the FITC-Dextran stock solution (**100 mg/ml**): Dissolve 100 mg FITC in 1 ml PBS in a 15 ml Eppendorf tube. Wrap tube in foil, vortex (mix) and incubate 4h at 4°C. This is the solution for the whole intestinal permeability assay (A). From this stock (100 mg/ml), prepare a **5 mg/ml** working solution by adding 50 ul of the stock to 950 ul PBS in a 1.5 ml cryovial tube. This is the solution for the intestinal transit time assay (B). Wrap in foil and store at -20°C until use.

> **NOTE:** FITC-DEXTRAN is sensitive to light and temperature. It should be protected from light (foil). It is stable for 6 months at -20°C and 3 weeks at 4°C.

2. PROCEDURE

Whole Intestinal Permeability:

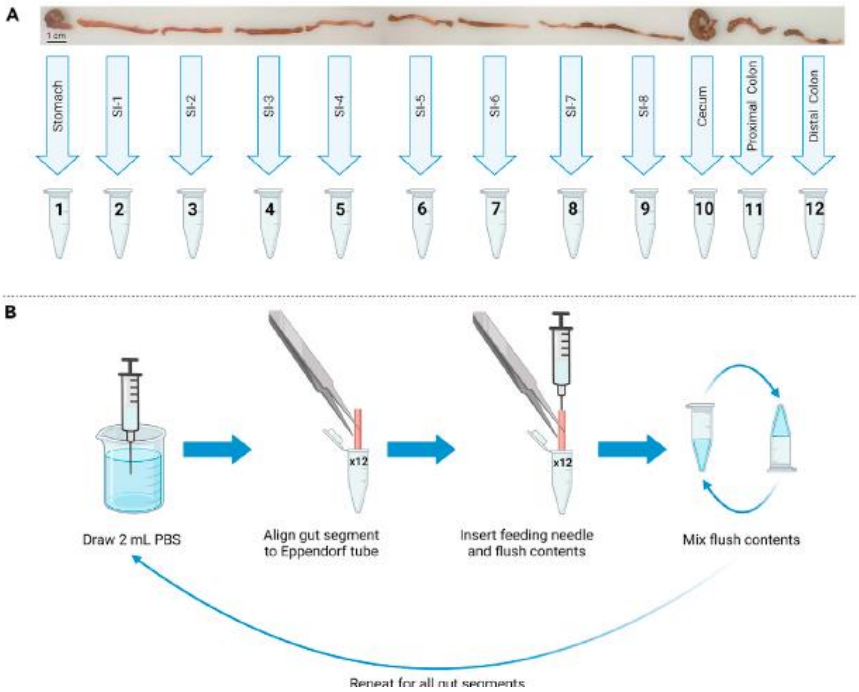
- **a.** Fast mouse for 6h prior the procedure.
- **b.** Gavage mouse with 200 µl of the **FITC working solution**.
- **c.** One hour after gavage, anesthetized the mouse using isoflurane (5% for induction, then set to 2% for blood draw).
- **d.** Draw at least 200 µl blood by retro-orbital bleed in lithium-heparin capillary tubes. Transfer to 1.5 ml Eppendorf tubes on ice.
- **e.** Centrifuge at 1500 x g for 15 min (at 4°C) to recover plasma.
- **f.** Determine FITC concentration in duplicates using a microplate spectrophotometer with 485 nm excitation and 535 nm emission.

Whole Intestinal transit time:

- **a.** Fast mouse for 6h prior the procedure.
- **b.** Gavage mouse with 200 µl of the **FITC working solution.**
- **c.** One hour after gavage, euthanized mouse by asphyxiation.
- **d.** Wet abdomen with 70% ethanol and cut a v-shape incision through the ventral abdominal wall from the pubis to the base of the ribcage.
- **e.** Remove the entire length of the small intestines and place – stretched on a paper tower.
- **f.** Using a clean razor blade, divide the entire GI tract into 12 segments, the stomach, the small intestine (divided into 8 segments, numbered from proximal to distal) and the colon (divided into cecum, proximal, and distal colon).
- **g.** Prepare a 5 ml syringe attached to a feeding needle.
- **h.** With forceps, pick up each segment and flush with 2ml cold PBS, into a 15 ml Eppendorf tube.
- **i.** Once all 12 segments are flushed, wrapped the collection tubes in foil and store at 4C.
- **j.** Determine FITC concentration in duplicates in black 96-well plates using a microplate spectrophotometer with 485 nm excitation and 535 nm emission.
- **k.** The percentage of total FITC fluorescence in the stomach and each intestinal segment can be calculated.

IMPORTANT NOTES:

Fluorescence should be determined as quickly as possible following the flush of the intestinal segments. Serial dilutions are usual needed for this assay. Perform a 1:10 dilution series as:
i. Aliquot 90 mL of 1X PBS into each empty well of columns 2–6 and 8–12.
ii. Then perform a serial dilution, starting with 10 mL in columns 1 and 7, and stopping at columns.
6 and 12, respectively. This is best performed using a multi-channel pipette.
iii. Remove 10 mL from 1:100,000 wells, columns 6 and 12.


A
1 cm
Stomach
SI-1
SI-2
SI-3
SI-4
SI-5
SI-6
SI-7
SI-8
Cecum
Proximal Colon
Distal Colon
1
2
3
4
5
6
7
8
9
10
11
12
B
x12
x12
Draw 2 mL PBS
Align gut segment to Eppendorf tube
Insert feeding needle and flush contents
Mix flush contents
Repeat for all gut segments