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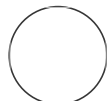
**Protocol status:** Working  
We use this protocol and it's working

**Created:** Dec 20, 2023

## Whole Gut Transit Time, Fecal Water Content, and Fecal Output

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### ABSTRACT

This protocol was used in "Peripheral Neuronal Activation Shapes the Microbiome and Alters Gut Physiology"

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Experiment Preparation		1d
1	6% (w/v) carmine red (Sigma-Aldrich, St. Louis, MO) with 0.5% methylcellulose (Sigma-Aldrich) was dissolved and autoclaved prior to use.	1d
Experiment		
2	Mice were administered C21 (3 mg/kg) intraperitoneally, and subsequently orally gavaged with 150 µL of carmine red solution.	5m
3	Following gavage, mice were single-housed with no bedding for the duration of the experiment, and animals were not fasted beforehand.	
4	Over the 5 hours following gavage, the time of expulsion was recorded for each fecal pellet, and each pellet was collected in pre-weighed, 1.5 mL microcentrifuge tube.	5h

- 5 Each pellet collected was checked for the presence of carmine red, and the time of initial carmine red pellet expulsion was recorded as GI transit time.

## Sample Processing

2d

- 6 The mass of collected fecal pellets was determined, and pellets were left to dry in an 80 °C oven for 2 days before weighing the desiccated pellets and calculating the pellets' initial water content.

2d

## Data Analysis

1h

- 7 Fecal output rate for each mouse was calculated as the total number of pellets expelled during the 5 hour time course post-C21 administration divided by the time the last fecal pellet was expelled.

1h