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# Fecal Carmine Red Protocol

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

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## Abstract

This assay is used to determine whole gut transit time. Mice are given an oral gavage containing a known volume of bright red carmine dye. Mice are placed into empty cages and are then observed in 15-minute intervals until they produce a bright red fecal pellet. The time between gavage and pellet expulsion is the time the dye takes to travel down the length of the GI tract, which is influenced by gastric emptying, and small and large intestinal transit.

## Materials

***Carmine Red Solution***
6% w/v carmine red (Sigma cat #C1022),
0.5% w/v methylcellulose (Sigma cat #M7027) in water.
***Sterile Cages***
***Standard Mouse Chow*** (whatever the mice currently are eating)
***Gavage needles***

## Troubleshooting

## Pre-protocol

1 Prepare Carmine Red Soln. (see materials)

2 Autoclave Carmine Red Soln. if sterility is required. (i.e. microbiome analysis)

3 Shake well or stir on low heat to homogenize

4 Once resuspended, immediately aliquot into 1.5 mL tubes and store in fridge to prevent spoilage.

5 Allow aliquots to come to room temperature prior to gavage.

6 Shake/vortex each right before gavage.

## Day of Set-up

1h

7 1.5 mL Bring mice to the testing room for at least 01:00:00 prior to oral gavage to acclimate.

1h

## Day of Assay

7h

8 Orally gavage mice with 100 µL carmine red solution. Generally, soft-tipped, disposable (Instec FTP-20-30, or similar) feeding needles, attached to a 1 mL slip-tip syringe.

Equipment

| | |
|---|---|
| Polypropylene Feeding Tubes for Rodents | NAME |
| Gavage Needle | TYPE |
| Instech Labs | BRAND |
| FTP-20-30 | SKU |
| https://www.instechlabs.com/products/feeding-tubes/polypropylene | LINK |

9 Record time of gavage for each mouse.

10 Following gavage, place animals back into home cage with cage mates, food, and water.

11 Allow animals to rest with food, water, in home cage for 01:00:00 (wildtype SPF mice will start producing red pellets 02:00:00 after gavage, while germ-free mice often take longer than 04:00:00 ). 7h

12 ·Split individual mice into single-housed, clean (or sterile, if needed) cages, with no bedding, which will interfere with the observation of a red fecal pellet. Cover with cage top.

13 Assay can be set up with or without food as long as all comparable runs are done the same way. If adding food, place 1-2 food pellets into a portion cup with water to create moistened chow (sterile if needed). Moistened chow reduces the risk of mice spilling water in the cage. Glass dishes can be used as cups to prevent flipping of the cups.

14 ***Optional:*** record number of pellets produced, cumulatively in 5-minute bins, for the first 30 minutes of separation (this can be informative of fecal output).

15 Every 00:15:00, check cages for a bright red pellet. Can observe through sides (with pen light if needed) or by opening cage top. Placing the cages on a white sheet of paper, rather than a
black benchtop, will also make visualization easier. If food was provided, check in the portion cups for red pellets as well. ***Ensure that whichever method of observation is used, all animals are disrupted similarly (ie. all animals have their cages opened).*** 15m

16 Pellets can be collected at each cage check, for water content or molecular assays, as long as
all cages are similarly disrupted during collection. Removing fecal pellets will also aid in determination of the first red fecal pellet produced.

17 Record time at which the first red pellet is observed for each mouse. For a normal, healthy adult mouse, this will be ~3-4hrs following gavage, but can range from 2-8+ hrs.

18 Once a red pellet is observed, return that mouse to its home cage.

19 To prevent stress associated with single-housing of mice, limit the single-housed portion of the assay to 6-8hrs, putting the maximum recordable transit time at 8-10hrs post-gavage.

20 If the maximum allotted time is reached without production of a red pellet, return the mouse to their
home cage, and record their transit time as the maximum.

## Analysis

21 Compare the "time to red pellet" for each genotype or treatment group. This can be done pre- and post- exposures for toxicity assessments.