

Keywords: Gastrointestinal transit, constipation, carmine red, motility

1. Prepare a sterile solution of carmine red (300  $\mu$ L; 6%; Sigma-Aldrich, Cat #C1022; St Louis, MO) suspended in 0.5% methylcellulose (Sigma-Aldrich, Cat #M0512; St Louis, MO).
2. Administer 0.3 mL of carmine red solution by gavage through a 21-gauge round-tip feeding needle
  - a. The time at which gavage takes place is recorded as  $T_0$ .
3. Place each mouse in separate cages without bedding.
4. One hour after gavage, fecal pellets were monitored at 10 min intervals for the presence of carmine red. Total GI transit time was considered as the interval between  $T_0$  and the time of first observance of carmine red in the stool.