TNBS protocol[[1](#_ENREF_1)]

Ethanol is used as a means to effectively disrupt intestinal barrier and enable the interaction of TNBS with colon tissue proteins. TNBS is a classical skin contactant serves as a hapten and it induced the Th1 inflammation, the colonic tissue infiltration by CD4 T cells and the secretion of various potent pro-inflammatory cytokines.

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| **Table 15.19.1** Mouse Strain Susceptibility to TNBS-Induced Colitis | | |
| Strain | Haplotype | Classification |
| ASW/Sn | *H-25* | Intermediate |
| Balb/c | *H-2d* | Susceptible |
| C3H/HeOU | *H-2K* | Intermediate |
| C57/B6 | *H-2b* | Resistant |
| C57/B10 | *H-2b* | Resistant |
| DBA/2 | *H-2d* | Resistant |
| SJL | *H-25* | Highly susceptible |

* Reagent

2,4,6-trinitrobenzenesulfonic acid

Ethanol

Inhalable anesthetic (methoxyflurane, isoflurane, halothane)

Surgical lubricant (e.g., Surgilube; E. Fougera)

* Other supplies

3.5-French(1/3mm) 38-cm, polyurethane catheter (Sherwood Medical)

1-ml disposable syringe

* Reagent and animal Preparing

1, Prepare an appropriate amount (0.5 to 4.0 mg) of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in 50% ethanol at a volume of 150 μl per mouse（protect from the light）.

Tips:100μl 2.5 mg/ml-50% ethanol -C57Bl/6J, 9-females, 10-weeks old, for 7 days[[2](#_ENREF_2)]

120μl 25mg/ml -50% ethanol -BALB/c, 8- male, 6-8 weeks old, for 10 days[[3](#_ENREF_3)]

2, Lightly anesthetize the mice by placing in a glass chamber containing inhalable anesthetic

Tips: *Anesthesia should be very light since prolonged anesthesia increases the contact time of the TNBS solution with the colon. Ideally anesthesia should not be so deep that vegetative parameters like breathing frequency are considerably decreased.*

*The mice do not need to be prepared in any fashion (e.g., bowel-cleansing enema, withholding food). However, some published paper mentioned food fasting from 12-24 h.*

3, Fill a 3.5-French, 38-cm, polyurethane catheter attached to a 1-ml disposable syringe with the TNBS mixture. Lubricate the catheter end by dipping the tip in surgical lubricant.

4, Hold the anesthetized mouse by the tail. Insert the catheter into the anus 0.5~1 cm and inject 50 μl TNBS mixture into the rectum. Advance the catheter carefully until a total of 3~4 cm is inserted in the colon and slowly inject an additional 100 μl TNBS mixture.

Tips: considering that there will be deaths during the injection.

5, Hold the mouse by the tail in a vertical position, head down, for 30~60 S to allow uniform distribution of the TNBS mixture before putting the animal back in its cage.

Tips: *An initial weight loss of 10% to 15% due to nonspecific toxic destruction of the mucosa is caused by the ethanol. The mice will recover in 2-3 days later, we need to set up a 50% ethanol control group.*

Refence

1. Scheiffele, F. and I.J. Fuss, *Induction of TNBS colitis in mice.* Current protocols in immunology, 2002. **49**(1): p. 15.19. 1-15.19. 14.

2. Jakobsson, T., et al., *The oxysterol receptor LXRβ protects against DSS-and TNBS-induced colitis in mice.* Mucosal immunology, 2014. **7**(6): p. 1416-1428.

3. Abad, C., et al., *Therapeutic effects of vasoactive intestinal peptide in the trinitrobenzene sulfonic acid mice model of Crohn's disease.* Gastroenterology, 2003. **124**(4): p. 961-971.