 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure LAB_075 Tail Suspension Test for Mice Institutional author: Queensland Brain Institute AEC Reviewed and Approved: March 2025 SOP Expiry: March 2026</p>	<p>Version #1</p> <hr/> <p>Page 1 of 3</p>
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LAB_075 Tail Suspension Test for Mice (Expiry: March 2026)

I. OBJECTIVE

To describe the procedure for measuring depression-related behaviour, and for screening of novel antidepressants or depression-inducing treatments in mice using the tail suspension test.

NB: The use of (*) indicates this statement is dependent on the facility procedures

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II. COMMENTS / RECOMMENDATIONS

- Behavioural assessments are ideally performed in a dedicated behavioural suite.
- The environment should be free from uncontrolled external stimuli that may influence the animal's behaviour such as human traffic, unnecessary noise, and intense lighting.
- Male and female rodents should be tested separately, with one sex in the room at a time. Where possible males should be tested first, preferably on separate days but with at least thorough cleaning between the sexes. This is unless rodents are already housed within wire top cages or equivalent and both sexes are present in the home room.
- The tail suspension test is generally considered to be a preferred alternative to the Forced Swim Test.
- **This test is not suitable for rats**

III. EQUIPMENT

- PPE*

Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.

- Appropriate trolley for transporting cages.
- Disinfectant* and paper towel for cleaning equipment.
- Apparatus – a simple setup of hooks suspended from a bar approximately 30cm above a bench top, with a screen separating each hook so that multiple mice can be tested simultaneously.



- Video recording equipment connected to a computer for video capturing.
To facilitate automatic tracking with video recording equipment, use diffuse lighting to minimise reflections.
- A curtain or screened off area for experimenter to be hidden from the mice during testing, if available.

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IV. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
2. Prepare equipment items including disinfecting prior to first use.
3. Bring rodents into the room (with lighting levels pre-set at the level required for the experiment) for at least 30 mins prior to start of experiment.

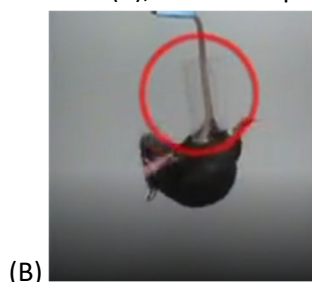
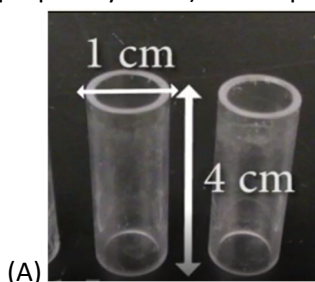
Length of habituation time in the testing room should be consistent for all rodents within an experiment.

V. PROCEDURE

1. Record light levels in the middle of the arena, for reproducibility and consistency.

Lux range should be between 20-900 LUX and should remain the same for all rodents within an experiment.

2. Handling of mice as per: [LAB_006 Handling and Restraint in Mice and Neonates](#)
3. If pharmaceutical substances need to be injected prior to test, follow AEC approved protocols (examples here):
[LAB_028 Injections - Intra-peritoneal \(IP\) in Mice, Rats and Neonates](#)
[LAB_017 Injections - Subcutaneous \(SC\) Injection in Mice and Rats](#)
4. Wrap adhesive tape around each mouse's tail in a constant position, three-quarters of the distance from the base of the tail to the tip. If a strain known to climb (e.g C57BL/6) their tails is used, a climb stopper (A) (lightweight perplex cylinder) can be placed onto their tails first (B), which will prevent climbing behaviour.



5. Start recording and identify subject/s within the camera view.
6. Suspend each animal by pushing the hook through the adhesive tape, close to the tail (1-2mm) to ensure the animal hangs straight down.
Ensure that you don't walk between the camera and mice already in position, as the test begins immediately.
7. Trials usually last 6 minutes. Initially, the mice will actively attempt to escape but will adopt more immobile postures the longer they are suspended.
8. At the end of the trial, carefully remove the animals from the hooks and gently remove tape from the tail before returning mice to their home cages.
9. Stop recording and make sure to save the video file.
10. Remove scat and thoroughly disinfect the maze and allow to dry completely.
11. Repeat testing of mice is possible e.g pre and post treatment, but some habituation (increased immobility) might occur.


VI. ANALYSIS

Analysis can be made manually from the video recording or using animal tracking software.

- Any mice observed trying to escape by climbing their tail (if not using a climb stopper) need to be excluded from the test. Mice that successfully climb their tails have learned that escape is possible. C57BL/6 mice are particularly prone to climbing behaviour.
- The main measure is the total time spent immobile over the full 6 min period. Small movements that are confined to the front legs but without the involvement of the hind legs should not be counted as

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mobility. Additionally, oscillations and pendulum like swings that are due to the momentum gained during the earlier mobility bouts also are not counted as mobility.

Analysis should be performed blind and consistent for all mice.

- Latency to first bout of immobility can also be measured. A bout is defined as a period of at least 1 sec without any escape behaviour.

VII. REFERENCES

1. Can, A., Dao, DT., Terrillion, CE. *et al.* The tail suspension test. *Journal of visualized experiments : JoVE* ,59 e3769. (2012). <https://doi.org/10.3791/3769>
2. Cryan, JF., Mombereau, C. & Vassout, A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience & Biobehaviour Reviews* **29**, 571-625 (2005). <https://doi.org/10.1016/j.neubiorev.2005.03.009>
3. Steru, L., Chermat, R., Thierry, B. *et al.* The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology* **85**, 367–370 (1985). <https://doi.org/10.1007/BF00428203>

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
#1	LBM	07/04/2022	07/04/2025

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