

LAB_073 Elevated Plus Maze Test for Rodents (Expiry: March 2026)

I. OBJECTIVE

To describe the procedure for measuring anxiety-related behaviour of rodents in an elevated plus maze.

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, intense lighting. Similarly, it is important that assessments are controlled for those stimuli which cannot be removed, such as time of day and light or dark phase.
- 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.

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.
- Arena – the elevated plus maze can be made of any material, which is painted matte, and consists of four arms (two open without walls and two enclosed by high walls). The recommended dimensions are specified in the table below.



Arena dimensions	Mouse	Rats
The length of the arms (cm)	25-40	40-60
The width of the arms (cm)	5-6	6-12
The height of the walls (cm)	15-25	25-35

Conditions:

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The height of the base of the maze (cm)	40-50	50-70
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- 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 rodents during testing, if available.

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. Start recording and identify subject/s within the camera view.
3. Handling of rodents as per: [LAB_006 Handling and Restraint in Mice and Neonates](#)
[LAB_039 Handling and Restraint in Rats and Neonates](#)
4. Place rodent at the junction of the open and closed arms, facing the open arm opposite to where the experimenter is.
5. Trials last anywhere from 5 minutes to 30 minutes** (when the effects of activating/silencing certain brain areas via optogenetic methods are being tested on anxiety-related behaviour or testing response to, or pharmacokinetics of anxiety drugs).
6. At the end of the trial, remove the rodent and return them to the home cage.
Ensure immediate access to food and water, particularly for longer trials.
7. Stop recording and make sure to save the video file.
8. Remove scat and thoroughly disinfect the maze and allow to dry completely.
9. Analysis can be made manually or using animal tracking software.
10. Repeat testing of rodents** in the elevated plus maze is possible with a minimum 3-week period between each test and with the arena moved to a novel room to prevent habituation to the test.

VI. ANALYSIS

Several different parameters can be analysed using an elevated plus maze. These include:


- Open or closed arm duration
- Open or closed arm entries
- The ratio of open or closed arm duration to the total arm duration
- The ratio of open or closed arm entries/time to the total arm entries/time
- Distance travelled and speed of travel
- Number of scat

VII. REFERENCES

1. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc.* 2007;2(2):322-328, doi:10.1038/nprot.2007.44. <https://www.nature.com/articles/nprot.2007.44>

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 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA</p> <p>CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure</p> <p>LAB_073 Elevated Plus Maze Test for Rodents</p> <p>Institutional author: Queensland Brain Institute</p> <p>AEC Reviewed and Approved: March 2025</p> <p>SOP Expiry: March 2026</p>	<p>Version #1</p> <hr/> <p>Page 3 of 3</p>
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2. Garcia AMB, Cardenas FP, Morato S. Effect of different illumination levels on rat behavior in the elevated plus-maze. *Physiol Behav.* 2005;85(3):265-70, doi: 10.1016/j.physbeh.2005.04.007.
<https://www.sciencedirect.com/science/article/pii/S0031938405001241?via%3Dihub>
3. Komada M, Takao K, Miyakawa T. Elevated plus maze for mice. *J Vis Exp.* 2008;(22):1088, doi:10.3791/1088
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2762911/>

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