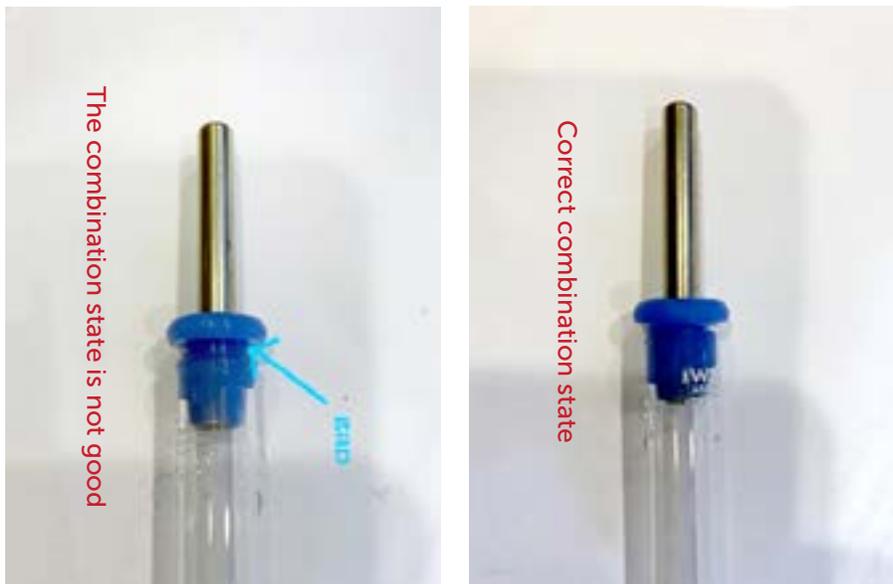


Drinko Measurer User's Guide & Sample Protocol:

To ensure that you are getting the best use out of the Drinko Measurer, we recommend a few simple tips for best practices:

1. The Drinko Measurer tube should fit tight and flush with the bottle.

If it seems too hard to fit the silicon cap all the way to the edge of the bottle opening, it is recommended to wet the silicon cap and bottle opening to make sure you achieve the appropriate fit.



2. For accurate fluid intake measurements, liquid should be weighed inside the bottles.

The silicon cap creates a very tight seal with the bottle to prevent leakage. However, there may be excess liquid remaining in the nozzle of the tube once the experiment is finished that could leak out if the tube was removed prior to weighing, therefore sacrificing measurements.

3. Thoroughly wash tubes and bottles after each use.

Note. We recommend doing one final rinse with deionized (DI) water after washing in order to remove any leftover residue that could impact wear over time. This is especially important for the ball bearings inside the nozzle.

4. Ensure that the clip on the tube is attached to the wiring of the cage top.

The Drinko Measurer is designed to prevent excess liquid leakage from the tube top when animals are not drinking. However, harsh movements of the bottle (i.e. if it is displaced or dislodged from its proper position on the cage top) could cause excess water to be dispelled from the nozzle tip.

Ex. Protocol : Measuring Capsaicin Intake (adapted from Kiode & Furuse, 2003)

Time (day)	Procedure accomplished	Age (weeks)	Capsaicin [μM]	Data Collected
-2 to 0	Mice are housed individually with continuous access to a water bottle (habituation period)	~8	0	-
1 to 3	Baseline water intake is measured for 12 hrs (20:00-8:00) during dark phase for 3 consecutive days	8-12	0	baseline intake of deionized water
4	Intake of 0.5 μM capsaicin solution is measured for 12 h during dark phase of one night using 1-choice bottle	8-12	0.5	amount of 0.5 μM capsaicin intake
5	Intake of 1 μM capsaicin solution is measured for 12 h during dark phase of one night using 1-choice bottle	8-12	1	amount of 1 μM capsaicin intake
6	Intake of 4 μM capsaicin solution is measured for 12 h during dark phase of one night using 1-choice bottle	8-12	4	amount of 4 μM capsaicin intake
7	Intake of 7 μM capsaicin solution is measured for 12 h during dark phase of one night using 1-choice bottle	8-12	7	amount of 7 μM capsaicin intake
8	Intake of 10 μM capsaicin solution is measured for 12 h during dark phase of one night using 1-choice bottle	8-12	10	amount of 10 μM capsaicin intake
9	Intake of 15 μM capsaicin solution is measured for 12 h during dark phase of one night using 1-choice bottle	8-12	15	amount of 15 μM capsaicin intake

Equipment

- 15 mL glass test-tubes capped with a silicon stopper with a double ball bearing nozzle (Drinko-measurer DM-G1 (O'Hara & Co. Ltd., Tokyo, Japan) (see Figure 1 below)
- Top-loading balance scale
- Ultrasonic bottle and nozzle cleaner

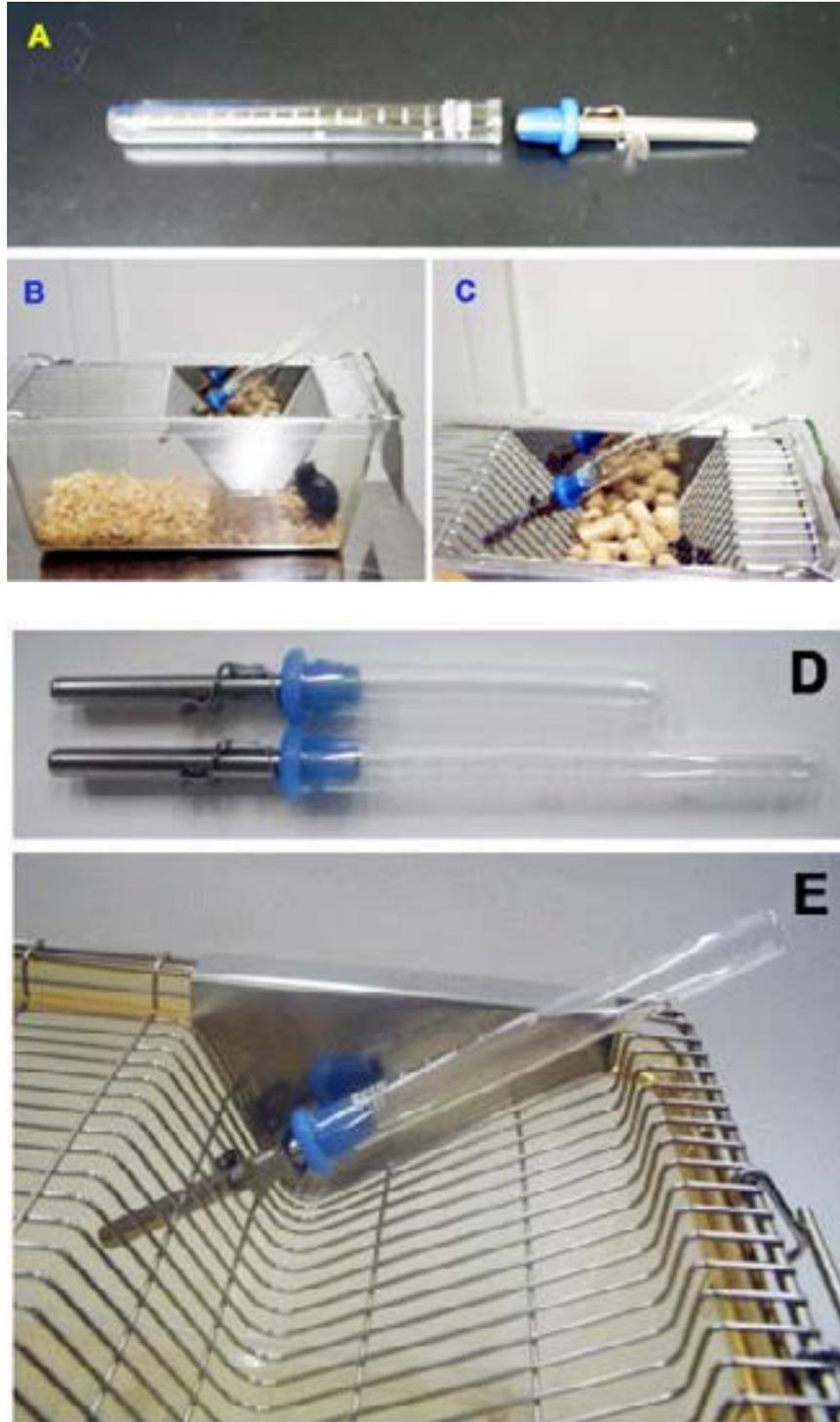


Figure 1. In Panels A & D, the 15 mL glass test-tubes are capped with silicon stoppers containing double ball bearing nozzles. In Panels B, C, and E, each test tube bottle is placed on the cage lid attached with a clip.

Reagents, supplies, solutions

- Capsaicin: (8-Methyl-N-Vanillyl-6-Nonenamide, minimum 97%, Sigma Chemical Co., St. Louis, MO)

1- Because capsaicin has low solubility in water, stock solution of capsaicin is prepared in ethanol (200 mmol/L).

2- The stock solution is then diluted with deionized water to 0.5, 1.0, 4.0, 7.0, 10.0, and 15.0 μM capsaicin concentration.

3- Ethanol concentration is adjusted and kept at 0.0075% in all capsaicin solutions.

- Ethanol (200 mmol/L)
- Autoclaved deionized water

Procedure for measuring consumption of capsaicin solution

a. Mice are individually housed and allowed to habituate for 2 days with deionized water their water bottle.

b. Before testing for capsaicin intake, baseline water intake is measured during the entire dark phase (20:00-8:00) for 3 days and the median volume is used as (100%) water intake volume.

c. Increasing concentration of capsaicin (0.5, 1.0, 4.0, 7.0, 10.0, and 15.0 μM) solution is then offered in the same 12-h 1-bottle test manner, each time with a new set of the same type of water bottle.

d. The volume of fluid intake during the entire 12-h dark phase is measured by weighing the test bottles before and after the test period and subtracting the final weight from the initial.

e. Used bottles and nozzles are washed with an ultrasonic cleaner following each measurement.

f. Capsaicin solution intake for each mouse is calculated as percent of the median intake of each mouse during the first 3 days of baseline (water only) measurement.

Submitting Investigator's Notes: "In order to examine the amount of fluid-loss during handling of bottles, we measured loss of fluid by doing the same type of handling using 50 different test bottles. Fluid-loss on average was around 0.013 mL, ranging from 0 to 0.061 mL. Because the amount of fluid-loss during handling of bottles was less than 1% of the fluid consumed by each animal in our test, we considered this source of error to be negligible."

Data collected by investigator

Fluid intake at increasing concentration of capsaicin (0.5, 1.0, 4.0, 7.0, 10.0, and 15.0 μM)