



## SPOT URINE COLLECTION PROTOCOLS FOR ALBUMIN AND CREATININE ASSAY

Version: 1  
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**Summary:** Collection of urine from experimental animals is a basic requirement to detect albumin/creatinine ratio for glomerular nephropathy. This is a non-invasive, simple, quick, and inexpensive method for collecting spot urine from mouse when a small volume of urine (10-500ul) sample is required.

### Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Customized spot urine cage- a clear plastic container for lean or obese mouse. 6 1/2" x 4 7/8" x 3 7/8" for lean mouse. (7 1/8" x 6 1/2" x 4 1/2" for obese mouse.	Amazon	SRERILITE 177718612 Flip Top Storage Box, Clear
SMALL PARTS, INC. Stainless Steel 304 Mesh #10, 0.023Wire Mesh Cloth Screen 12"x24"	Amazon	N/A
RAFTSMAN 3/8 INCH Electric Drill and 1/8 INCH Drill Bit	Sears	N/A
Metal Cutter and Flyer from	Home Depot	
Paper towels, disinfectant spray, anti-bacterial soap, cleaning brush and sponge	Fisher Scientific	Supplied by Lab. Animal unit

## Protocol:

***WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.***

1. Create 6 evenly spaced holes in the lid of the container – 3 on top and 3 at bottom using an electric drill using 1/8” drill bit. Also drill 2 holes each on four sides of the container. Using a metal cutter cut out a 14.5 cm by 9.5 cm piece of stainless steel wire mesh to create a base for mouse that will fit inside the lean mouse cage. Then bend all four sides of the wire mesh using a flyer to create 1 cm perpendicular bent on each sides. Insert the wire mesh base inside the container with the side bents pointing downward. The base should have a snug-fit inside the container about 1” above the bottom. Create similar way for obese mouse cage. Wire mesh need to be washed and autoclaved before using.
2. It is important to acclimatize the mouse in the metabolic cage for about half hour to one hour every day for 3 consecutive days before starting urine collection.
3. Prepare appropriate number of urine collecting tubes by labeling a tube with identification number for each animal.
4. To maintain SPF housing, place cages in an operational laminar flood hood.
5. Place the appropriate mouse in the metabolic cage and replace the cage lid. Be certain the lid is secure. Put one mouse per box and label the top of the box with the mouse number.
6. Place the mouse cage card in front of metabolic cage. Make sure mouse cage card has all the necessary information such as mouse identification number, mouse protocol number, emergency contact number, Principal Investigator name, date, and time period of 4 hour urine collection, also “DO NOT FEED” sticker (Provided by Lab Animal Facility)
7. Because mice need to be left without food or water for 4 hours, an exception must be completed.
8. Collect urine for 4hour period without food and water access.
9. Check on mice every hour to monitor mouse health conditions.
10. After 4hour remove the mouse from the metabolic cage and replace in the original housing cage and carefully collect the urine.
11. Collect urine using a pipette (P200 is usually best) and place in a 1.5 mL micro centrifuge tube.
12. Spin urine at 2000 rpm for 5 min. Remove supernatant and place in a clean 1.5 mL tube.
13. Store urine samples at 4<sup>0</sup>C until ready for assay in short period and at -20<sup>0</sup>C or -80<sup>0</sup>C for a longer period.
14. Clean metabolic cage with appropriate disinfectants. Hot, soapy water may be used on the acrylic parts. All cage parts should be sprayed with a bactericidal,

virucidal, and fungicidal (e.g., Clidox® or Spor-Klenz ®) and wiped down. Dry each part and reassemble

**Reagent Preparation:** protections e.g. Safety glasses, gloves, lab coat.

Reagent 1: N/A

Reagents and Materials:

Procedure: Careful during working with Metal cutter, electric Drill, Stainless Steel meshes.

Use lab personnel protections e.g. Safety glasses, gloves, lab coat

Checklist: N/A