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Working

U Michigan - Glomerular Filtration Rate Determination with Minipump Inulin Clearance

Jeff Hodgin[1]

[1]University of Michigan - Ann Arbor

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Mouse Metabolic Phenotyping Centers
Tech. support email: **info@mmpc.org**

Lili Liang

ABSTRACT

Summary:

This is the protocol for measuring Glomerular Filtration Rate (GFR) with minipump FITC-inulin clearance in mice. In brief, Alzet micro-osmotic pumps (minipumps, Model 1007D) are filled with 3% FITC-inulin solution. These pumps release FITC-inulin at a rate of 0.5 µl/hr at least seven days. Mouse is temporarily anesthetized and then two mini-pumps are implanted in mouse peritoneal cavity through a tiny midline incision. After surgery, 24-hour urine is collected with metabolic cage and 100 µl of blood from saphenous vein is obtained at the same time. The concentrations of FITC-inulin in urine and blood samples are measured by flurometer and GFR is calculated by the concentration of FITC-inulin in 24-hour-urine and the concentration of FITC-inulin in plasma.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=310&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
FITC-inulin	F3237	Sigma Aldrich		
Minipumps	Model 1007D	Alzet		

MATERIALS TEXT

Reagent Preparation:

Reagent 1:

Reagents and Materials
1. 0.85% NaCl: add 8.5 g NaCl into 1 L ddH_2O.
2. FITC-inulin
3. Dialysis membrane
4. Syringe filter

Procedure

1. Weigh FITC-inulin and dissolve in 0.85% sodium chloride solution by heating to 90°C until completely dissolved to prepare 3 % FITC-inulin solution.

2. Measure the weight of dissolved FITC-inulin solution.

3. Get a 20 cm piece of dialysis membrane (molecular weight cut-off: 1,000) and balance in ddH_2O for 30 min and then wash a few times.

4. Fill dissolved FITC-inulin into dialysis membrane and seal tightly.

5. Measure the weight of dialysis membrane.

6. Insert the dialysis membrane in 1 L 0.85% sodium chloride solution and stir with light-protected for 24 hours at Room Temperature, water will osmotically move into the membrane and unbound FITC, or bound FITC-inulin <1,000 molecular weight will move out of the membrane.

7. Determine the weight of dialysis membrane again.

8. Calculate the final concentration of FITC-inulin (C): C = n/V, n = initial FITC-inulin amount, V = new volume (difference in weight of dialysis tubing before and after dialysis plus volume of initial FITC-Inulin solution).

9. Filter FITC-inulin with a 0.22 µm syringe filter before injecting to the osmotic pumps.

10. Protect FITC-inulin from light all the time with aluminum foil at 4°C. Dialyzed and sterilized FITC-inulin can be used for up to 2 weeks. Participated FITC-inulin can be dissolved by re-heating at 90°C for a few minutes.

Note:
Sigma-Aldrich RRID:SCR_008988

1 Minipump preparation:

The micro-osmotic pumps are filled with approximately 100 µl of a 3 % FITC inulin solution.

2 Procedures of surgery:

2.1 Mice are anesthetized with isoflurane in gas anesthetic machine rented from Unit for Laboratory Animal Medicine (ULAM), University of Michigan.

2.2 Two minipumps are inserted into peritoneal cavity of mouse through an approximate 5 mm length of abdominal midline incision.

3 Blood and urine sample collection:

3.1 After the recovery period, mice are placed into metabolic cages and 24-hour urine is collected.

3.2 Immediate after urine sample collection, mice are restrained inside a 50-mL centrifuge tube with air-holes drilled in the tip.

3.3 The inner thigh is closely shaven and wiped with 70% ethanol, revealing the saphenous vein. A small incision is made with a scalpel, and 100-µl-blood is collected with a heparinized capillary tube (Fisher Scientific). The blood sample is centrifuged at 4000 rpm for 10 min and then take out plasma for the latter measurement.

4 Measurement of inulin in plasma and urine:

4.1 The urine samples are diluted 1:5 and 1:10 in HEPES, respectively.

4.2 The plasma samples are mixed with HEPES (pH 7.4) by 4:1.

4.2 The processed samples are loaded onto a 96-well plate (COSTAR 3595), 50µl of sample/well.

4.3 The fluorescence is measured with Fluoroscan Ascent FL (Labsystems), at excitation 485 nm, and emission 530 nm.

4.4 Standard curve: For each point on the urine standard curve, 200 µl of normal mouse urine is diluted into 300 µl HEPES. Then, 500 µl of FITC-inulin solution of varying known concentrations (dissolved in HEPES) is added to each tube. Five points are included on the urine standard curve, with inulin concentrations ranging from 0.05 mg/ml to 0.0008 mg/ml. For each point on the plasma standard curve, 160 µl of normal mouse plasma are mixed with 40µl of an inulin solution (made in HEPES) of known concentration. Four points are included on the plasma standard curve, with inulin concentrations ranging from 0.005 mg/ml to 0.0006 mg/ml. Then the standard curves are used to determine the concentration of FITC-inulin in the samples.

5 Calculation of GFR:

GFR is calculated by the concentration of inulin excreted in urine divided by the concentration of plasma inulin and expressed in ml/min/g

body weight.