



## Basal glucose metabolism

Version: 1

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*(note that the following list should be linked to the appropriate location.)*

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**Summary:** *(This area will include a brief description of what the protocol is used for and why someone would need to use it.)*

Whole body glucose turnover and hepatic glucose production rates are measured at basal state using an intravenous infusion of labeled glucose in awake mice. Whole body glucose turnover and hepatic glucose production regulate basal glucose levels and are altered in obesity.

**Reagents and Materials:** *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	Vendor	Stock Number
[3- <sup>3</sup> H] D-glucose	Perkin Elmer	NET331C005MC
0.9 % Sodium Chloride, Injection, USP	B.Braun Medical Inc	NDC0264-4001-55
Pentobarbital	Oak Pharmaceuticals, Inc.	NDC76478-501-50
Barium hydroxide monohydrate (0.3 N)	Sigma	B4059
Zinc sulfate heptahydrate (0.3 N)	Sigma	Z2876
Scintillation cocktail	PerkinElmer	6013329
Beckman Coulter LS6500 Multi-Purpose Scintillation Counter	Beckman	LS6500

### Protocol:

1. Survival surgery is performed to establish a chronic indwelling catheter at 5~6 days prior to experiment for intravenous infusion. (refer to M1023: Surgery-jugular vein cannulation)
2. Mice are fasted overnight (~15 hours) or for 5 hours prior to the start of experiment.
3. Place a mouse in a rat-size restrainer with its tail tape-tethered at one end.

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4. Expose and flush the intravenous catheter using saline solution. Then, connect the catheter to the CMA Microdialysis infusion pump.
5. Collect plasma sample (10  $\mu$ l) before the start of infusion (basal-0 min) to measure basal glucose levels.
6. Start the experiment by turning on the pump and intravenously infusing [ $3\text{-}^3\text{H}$ ] D-glucose at 0.05  $\mu\text{Ci}/\text{min}$  in awake mice. ([ $3\text{-}^3\text{H}$ ] D-glucose is suspended in saline solution)
7. Collect plasma samples (10  $\mu$ l each) at 90, 100, 110, and 120 min to measure glucose levels.
8. Collect additional plasma samples (10  $\mu$ l each) at 90, 100, 110, and 120 min to measure [ $3\text{-}^3\text{H}$ ] D-glucose concentrations. (10  $\mu$ l plasma samples are suspended in 20  $\mu$ l distilled water [ $\text{dH}_2\text{O}$ ] to make 30  $\mu$ l sample solutions.)
9. At the end of experiment, mice are euthanized using pentobarbital.
10. Biochemical assay is conducted using plasma samples to measure [ $3\text{-}^3\text{H}$ ] D-glucose concentrations.
  - a) Transfer 15  $\mu$ l of plasma sample solutions into microcentrifuge tubes with sample time clearly labeled.
  - b) Add 25  $\mu$ l BaOH and vortex samples.
  - c) Add 25  $\mu$ l  $\text{Zn}(\text{SO})_2$  and vortex samples.
  - d) Centrifuge samples for 5 min at 12,000g (~14,000 rpm).
  - e) Transfer 20  $\mu$ l of supernatant into scintillation vials and place into vacuum oven set at room temperature for overnight drying.
  - f) Following overnight drying, add 80  $\mu$ l  $\text{dH}_2\text{O}$  and vortex thoroughly.
  - g) Add 3 ml of Ultima scintillation cocktail and vortex sample.
  - h) Measure [ $3\text{-}^3\text{H}$ ] D-glucose using Beckman Coulter Scintillation Counter.
11. Basal rate of whole body glucose turnover is calculated as the ratio of the [ $^3\text{H}$ ]glucose infusion rate to the specific activity of plasma glucose averaged for 90~120 min of experiment.

## Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

[Reagent 1](#)

[Reagent 2](#)

[Reagent 3](#)

Reagent 1: [ $3\text{-}^3\text{H}$ ] D-glucose infusion solution

### Reagents and Materials

1. [ $3\text{-}^3\text{H}$ ] D-glucose
2. 0.9 % Sodium Chloride, Injection, USP

### Procedure

1. Transfer [ $3\text{-}^3\text{H}$ ]glucose into a glass tube.
2. Place [ $3\text{-}^3\text{H}$ ]glucose solution in a vacuum oven set at room temperature for 2 days for vacuum drying of ethanol.
3. Re-suspend [ $3\text{-}^3\text{H}$ ]glucose using saline for intravenous infusion.