



May 09,
2019

Working

U Mass - Basal glucose metabolism [↗](#)

Jason Kim¹

¹University of Massachusetts

dx.doi.org/10.17504/protocols.io.xr8fm9w

Mouse Metabolic Phenotyping Centers

Tech. support email: info@mmpc.org

Lili Liang

ABSTRACT

Summary:

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.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=137&docType=Protocol>

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
[3-3H] D-glucose	NET331C005MC	Perkin Elmer		
0.9 % Sodium Chloride Injection USP	NDC0264-4001-55	B.Braun Medical Inc		
Pentobarbital	NDC76478-501-50	Oak Pharmaceuticals, Inc.		
Barium hydroxide mono- hydrate (0.3 N)	B4059	Sigma-aldrich		
Zinc sulfate heptahydrate (0.3 N)	Z2876	Sigma-aldrich		
Scintillation cocktail	6013329	Perkin Elmer		
Beckman Coulter LS6500 Multi-Purpose Scintillation Counter	LS6500	Beckman Coulter		

MATERIALS TEXT

Reagent Preparation:

Reagent 1: [3-³H] D-glucose infusion solution

Reagents and Materials

1. [3-³H] D-glucose
2. 0.9 % Sodium Chloride, Injection, USP

Procedure

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

Note:

Sigma-Aldrich [RRID:SCR_008988](#)

B Braun Medical, Cite this (B Braun Sharing Expertise, [RRID:SCR_007148](#))

Beckman Coulter, [RRID:SCR_008940](#)

- 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.
- 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 µl) 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 [3-³H] D-glucose at 0.05 µCi/min in awake mice. ([3-³H] D-glucose is suspended in saline solution)
- 7 Collect plasma samples (10 µl each) at 90, 100, 110, and 120 min to measure glucose levels.
- 8 Collect additional plasma samples (10 µl each) at 90, 100, 110, and 120 min to measure [3-³H] D-glucose concentrations. (10 µl plasma samples are suspended in 20 µl distilled water [dH₂O] to make 30 µl sample solutions.)
- 9 At the end of experiment, mice are euthanized using pentobarbital.
- 10 Biochemical assay is conducted using plasma samples to measure [3-³H] D-glucose concentrations.
 - a) Transfer 15 µl of plasma sample solutions into microcentrifuge tubes with sample time clearly labeled.
 - b) Add 25 µl BaOH and vortex samples.
 - c) Add 25 µl Zn(SO)₄ and vortex samples.
 - d) Centrifuge samples for 5 min at 12,000g (~14,000 rpm).
 - e) Transfer 20 µl of supernatant into scintillation vials and place into vacuum oven set at room temperature for overnight drying.
 - f) Following overnight drying, add 80 µl dH₂O and vortex thoroughly.
 - g) Add 3 ml of Ultima scintillation cocktail and vortex sample.
 - h) Measure [3-³H] D-glucose using Beckman Coulter Scintillation Counter.

Basal rate of whole body glucose turnover is calculated as the ratio of the [³H]glucose infusion rate to the specific activity of plasma glucose

11 averaged for 90~120 min of experiment.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited