



# D4004 Digestible Energy by Bomb Calorimetry

Version: 2

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**Summary:** Changes in fecal energy loss can result in differences in net energy balance and feed efficiency, and may result from, e.g., alterations in bile acid production, secretion or re-uptake, and changes in activities of enzymes involved in macronutrient digestion or uptake (e.g., pancreatic lipase). This assay will be coupled to food intake determinations and will involve fecal collection and feces weight measures for 48h, which can be coordinated with the meal pattern studies (catalog item D4003) or indirect calorimetry (catalog item D4007). Diet and fecal energy content will be measured by bomb calorimetry and the following reported: fecal energy density, total fecal energy loss/day, and fecal energy loss as a percent of energy intake. Alternatively, fecal samples and diet samples can be examined without food intake measurements. In this case, only diet and fecal energy density will be reported. Additional tests can be done through proximate analysis (D4005- Digestible Energy by Proximate Analysis) to determine protein or fat absorption.

## Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Tweezers		
Disinfectant solution (70% isopropanol or 70% ethanol)		
Labeled pre-weighed 1.5 ml tubes		
Gloves		
Small weigh boats		
Measuring cup/beaker for food weight measurements		
Large beaker for mouse weight measurements		

## Protocol:

1. Mice are singly housed in clean cages with fresh food and water and low bedding.

*IMPORTANT: Special consideration should be given to the type of bedding used during the 48 hour collection period. When bedding is used, we recommend Carefresh Bedding (Absorption Corp, #868744), using slightly less than the normal amount of bedding per cage (low bedding conditions). For the short collection period, no bedding or special raised cage bottoms will simplify the fecal collection process; however, these modifications will require IACUC amendment approval. Also, corncob bedding (i.e. Bed-O'Cobs, 1/8in; Andersons Lab 8B) is not advised due to the difficulty in sifting through it to collect feces and the fact that it has caloric value (which can complicate analyses- the mice may eat it).*

2. Weigh mouse and “food-in” (grams of rodent diet provided at start of experiment).
3. Save several pellets of rodent diet for analysis (e.g., for energy or macronutrient content)-Store at 4°C in airtight container.
4. At 24h, weigh mouse and “food-out” (grams of rodent diet remaining)
5. At the end of 48h collection period, weigh mouse and “food-out”.
6. Swap out the cage bottoms with new clean bottoms, replace feeder insert/water bottle and return animals to housing system.
7. For each cage, carefully inspect and remove each piece of bedding using tweezers to collect all feces. Inspect each pellet of feces to assure that there is no spilled food or fibers of hair or bedding attached. If significant food spillage is found in cage bottom, weigh in pre-tared weigh boat to add back to food measurement.
8. Place feces in labeled pre-weighed 1.5 ml tube.
9. Weigh tubes and record weight.
10. Subtract this value from original tube weight to determine fecal “wet” weight.
11. After collection, fecal samples are stored at -80C until analysis for bomb calorimetry.

**Data collected:**

48h food intake  
48h fecal “wet” weight  
fecal energy density  
total fecal energy loss/day  
fecal energy loss as a percent of energy intake  
breakdown of fecal energy loss: protein, fat, carbohydrate

Enclose fecal/diet sample log with samples and send on dry ice to:

Mouse Biology Program  
C/O UC Davis MMPC  
University of California  
2795 Second Street, Suite 400  
Davis, CA 95618  
530-757-3333

Please include MBP/MMPC project # in reference section on package documentation and send the FedEx/UPS tracking number information to: [mmpc-bodyc@ucdavis.edu](mailto:mmpc-bodyc@ucdavis.edu)