



Gross Body Composition (DEXA)

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Summary:

Rapid and accurate measures of fat and lean mass will be carried out in isoflurane-anesthetized mice, using a PixiMus dual energy X-ray absorptiometry (DEXA) device. This approach is especially attractive when it is desirable to utilize the same mice for several phenotypic measurements (e.g., when relatively few mice of a particular genotype are available), and measurements may be taken within ~5 min/mouse. Total bone mineral density (BMD, in gm/cm²) and bone mineral content (BMC, in mg.) values are obtained automatically from the DEXA scan and will be provided to MMPC clients as part of the dataset. Regional determinations of BMD and BMC can also be provided for a nominal data processing fee by utilizing the region-of-interest (ROI) capability of the PixiMus software. When terminal studies are conducted, whole-animal DEXA data can be complemented by determinations of adiposity (catalog item D4002) by dissection and measurement of adipose depot-specific weights (summed weights of gonadal, retroperitoneal, and femoral subcutaneous fat depots divided by live weight.)

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Piximus densitometer	GE Medical Systems, LUNAR	
Lunar Piximus trays	GE Medical Systems	30950
Phantom mouse for calibration	GE Medical Systems	
Anesthetic (Isoflurane)	Fisher Scientific	NC9259743
Vaporizer	VetEquip	
Medical grade O ₂	Airgas	OX USPEA
Caliper		
Analytical Scale		

Protocol:

Caution: Radiation - Exposure to X-rays

Exposure is minimized by the use of the special lead glass shielding cabinet of the machine. Please ensure that the door is firmly closed during the scan.

There are 4 main parts to this procedure: 1. Setting up the PIXImus analyzer, 2. Preparing the mouse, 3. Performing the measurements and monitoring animal recovery, 4. Processing the image data and performing calculations.

Setup:

1. Turn on Piximus at least 2 hours prior to start of 1st scan.
2. Open Piximus software and initiate calibration using “phantom” (an object with defined bone mineral density and fat content for unit calibration).

NOTE: This step requires approximately 1 hr to complete.

Prepare animal

1. Anesthetize animal using 2-4% isoflurane in O₂ by inhalation.
2. Monitor animal – Depth of anesthesia will be assessed by response to toe and tail pinch, with lack of response indicative of adequate anesthesia.
3. Weigh animal on analytical scale and record measurement.
4. Measure length of animal with calipers from the tip of the nose to the beginning of the tail (anus) to the nearest 0.5 cm., assuring that the animal’s spine is fully extended (gentle pull). Record measurement.

Perform Measurements

1. Place unconscious mouse on the specimen tray on the DEXA analyzer.

IMPORTANT: Body position is critically important for consistent and valid analysis.

Mice should be positioned with the legs slightly outstretched and the soles of the feet down as best possible; the tail may be included by curling the tail around left side of mouse toward the head or it can be left straight and excluded from the analysis. Make sure the spine is in a straight line, ensuring that the entire mouse is within the outlines of the sticky pad. Its nose should be positioned within the nosecone (head to the left as looking at the machine). The mouse should be as flat as possible with the spine fully extended.

2. Enter subject ID, body weight, gender, date of birth, and body length measurements and start scan. When the first image of the mouse appears on the computer screen, ensure it is positioned correctly for the scan, with all limbs and body proper in the field. If not, stop the run, re-position the mouse, and re-start.
3. While the 1st mouse is being scanned, you will want to start to anesthetize the next mouse. The DEXA scan will take about 4-5 minutes to run the full scan.
4. Once scan is complete, return mouse to its home cage and monitor its recovery over 1 hr.

Image Processing/Calculations/Animal Recovery

1. At a later time, the image files can be processed to exclude the head region. Within the Piximus software, this is accomplished by adjusting the placement of the green circle which removes the region from analysis. The scan data is then reprocessed and can be exported for further analysis. The standard data reported are shown below

<u>Data collected</u>	<u>units</u>
Body Composition	(% fat)
Lean and Fat tissue mass	(g)
Bone mineral density (BMD)	(g/cm ²)
Bone mineral Content (BMC)	(mg)
Bone area	(cm ²)

Note: A specific region of interest (ROI) can be manually selected within the total body image using the Piximus software (red box) to obtain specific localized measurements.

Bone area measurement is generated by outlining or specifying the limits or dimensions of the entire skeletal bone regions of the body (limbs, neck, spine, and tail), excluding the head, as regions of interest (ROI) following a full body X-ray scan.

Bone mineral content (BMC) is generated from PIXImus density scans which are assessed for accuracy using a set of 0.0 mg to 2,000 mg of hydroxyapatite standards.

BMD = BMC ÷ bone area

Lean body (non-fat) tissue weight = (Total body tissue weight – body fat weight)

Body fat tissue weight = (Total body tissue weight - lean body tissue weight)

Total body tissue weight = (BMC + Body fat tissue weight + Lean body tissue weight)

Representative image (screen shot) of an analysis showing excluded head region (green oval) and in this case, a region of interest for bone parameter (ROI, as red rectangle). Results are presented to the right as “TOTAL” whole body or within the “ROI.”

