

Assessing Energy Substrate Oxidation *In Vitro* with $^{14}\text{CO}_2$ Trapping

 Chao Song^{1,2}, Arianna Valeri¹, Fanxin Long¹
¹Translational Research Program of Pediatric Orthopedics, Department of Surgery, The Children's Hospital of Philadelphia ²Department of Orthopedic Surgery, Tongji Hospital, Huazhong University of Science and Technology

Corresponding Author

Fanxin Long

longf1@email.chop.edu

Citation

 Song, C., Valeri, A., Long, F. Assessing Energy Substrate Oxidation *In Vitro* with $^{14}\text{CO}_2$ Trapping. *J. Vis. Exp.* (181), e63568, doi:10.3791/63568 (2022).

Date Published

March 23, 2022

DOI

10.3791/63568

URL

jove.com/video/63568

Materials

Name	Company	Catalog Number	Comments
0.22 μm filters	Sigma-Aldrich	SLGVM33RS	Used to filter BSA solution
0.25% Trypsin-EDTA	Gibco	25200056	Dissociate cells from cell culture plates
1.5 mL Eppendorf tubes	PR1MA	PR MCT17 RB	Used for reaction incubation
10 cm plates	TPP	93100	Used for cell culture
10 mL syringe	BD	302995	Used to flush marrow from long bones
10% FBS	Atlanta biologicals	S11550	For Cell culture medium preparation
^{14}C -Glucose	PerkinElmer	NEC042X050UC	Used to make hot media
^{14}C -glutamine	PerkinElmer	NEC451050UC	Used to make hot media
^{14}C -oleate	PerkinElmer	NEC317050UC	Used to make hot media
23 G needle	BD	305120	Used to flush marrow from long bones
24-well plates	TPP	92024	Used for cell culture
70 μm cell strainers	MIDSCI	70CELL	Used to filter supernatant during cavalial digestion
Acridine Orange/Propidium Iodide (AO/PI) dye	Nexcelom Biosciences	CS2-0106	Stains live cells to determine seed density
Bovine Serum Ablumin	Proliant Biologicals	68700	Used for fatty acid conjugation
Cellometer Auto 2000	Nexcelom Biosciences		Determine the number of viable cells
Centrifuge	Thermo Fisher	Legend Micro 21R	Used to pellet cells
Collagenase type II	Worthington	LS004176	Dissociate cells from tissue
Custom MEM alpha	GIBCO	SKU: ME 18459P1	Used to create custom hot media
Dulbecco's Phosphate-Buffered Saline	Gibco	10010023	Used to dissolve and dilute reagents, and wash culture dishes
Filter Paper	Millipore-Sigma	WHA1001090	Traps CO_2 with sodium hydroxide
Glucose	Sigma-Aldrich	g7528	Used to make custom media
HEPES	Gibco	15630080	Traps CO_2 during cell culture

L-carnitine	Sigma-Aldrich	C0283	Supplemented for fatty acid oxidation
L-Glutamine	Sigma-Aldrich	g3126	Used to make custom media
MEM alpha	Thermo	A10490	Cell culture medium
Parafilm	Pecheney Plastic Packaging	PM998	Used to seal cell culture dishes
Penicillin-Streptomycin	Thermo Fisher	15140122	Prevents contamination in cell culture
Perchloric Acid	Sigma-Aldrich	244252	Releases CO ₂ during metabolic assay
Pyruvate	Sigma-Aldrich	p5280	Used to make custom media
Scintillation Counter	Beckman Coulter	LS6500	Determines radioactivity from the filter paper
Scintillation Fluid	MP Biomedicals	882453	Absorb the energy emitted by RAMs and re-emit it as flashes of light
Scintillation Vial	Fisher Scientific	03-337-1	Reaction containers for scintillation fluid
Sodium carbonate	Sigma-Aldrich	S5761	Balance buffer for medium
Sodium Hydroxide	Sigma-Aldrich	58045	Traps CO ₂ during metabolic assay
Sodium oleate	SANTA CRUZ	SC-215879	BSA conjugated fatty acid preparation
Vacuum filtration 1000	TPP	99950	Filter cMEMa