

Materials List for:

Measuring Liver Mitochondrial Oxygen Consumption and Proton Leak Kinetics to Estimate Mitochondrial Respiration in Holstein Dairy Cattle

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Materials

Name	Company	Catalog Number	Comments
Liver Biopsy			
Equipment			
Schackelford-Courtney bovine liver biopsy instrument	Sontec Instruments Englewood CO	1103-904	
Suture	Fisher Scientific	19-037-516	
Suture needles	NA	NA	Included with Suture
Scalpels	Sigma - Aldrich	S2896 / S2646	# for handle and blades
Surgery towels	Fisher Scientific	50-129-6667	
Falcon tubes 50 mL	Fisher Scientific	14-432-22	
Tweezers	Sigma - Aldrich	Z168750	
50 mL syringes	Fisher Scientific	22-314387	
Injection needles (22, 2 1/2)	VWR	MJ8881-200342	
Cow halter	Tractor Supply Co.	101966599	
Cotton swabbing	Fisher Scientific	14-959-102	
cotton gauze squares (4x4)	Fisher Scientific	22-246069	
Medical scissors	Sigma - Aldrich	Z265969	
Chemicals			
Coccidiosis Vaccine 0.75 bottle/ cow			Provided by Veterinarian
Clostridia Vaccine			Provided by Veterinarian
Liver biopsy antibiotics excenel 2 cc/100 lbs for 3 days			Provided by Veterinarian
Providone Scrub	Aspen Veterinary Resources	21260221	
Ethanol 70%	Sigma - Aldrich	793213	
Xylazine hydrochloride 100 mg/ mL IV at 0.010-0.015 mg/kg bodyweight			Provided by Veterinarian
2% lidocaine HCl (10-15 mL)			Provided by Veterinarian
1 mg/kg IV injection of flunixin meglumine			Provided by Veterinarian
Isolation of Mitochondria (liver)			
Equipment			
Wheaton vial 30 mL with a Teflon pestle of 0.16 mm clearance	Fisher Scientific	02-911-527	
Homogenizer Motor	Cole Parmer	EW-04369-10	

Homogenizer Probe	Cole Parmer	EW-04468-22	
Auto Pipette (10 mL)	Cole Parmer	SK-21600-74	
Beaker (500 mL) with ice	Fisher Scientific	FB100600	
Refrigerated microfuge	Fisher Scientific	75-002-441EW3	
Microfuge tubes (1.5 mL)	Fisher Scientific	AM12400	
Chemicals			
Bicinchoninic acid (BCA) protein assay kit (microplates for plate reader)	abcam	ab102536	
Sucrose	Sigma - Aldrich	S7903-1KG	
Tris-HCl	Sigma - Aldrich	T1503-1KG	
EDTA	Sigma - Aldrich	EDS-1KG	
BSA (fatty acid free)	Sigma - Aldrich	A7030-50G	
Mannitol	Sigma - Aldrich	M4125-1KG	
Deionized water	Sigma - Aldrich	38796	
Hepes	Sigma - Aldrich	H3375-500G	
Use to create mitochondria isolation media: 220 mM mannitol, 70 mM sucrose, 20 mM HEPES, 20 mM Tris-HCl, 1 mM EDTA, and 0.1% (w/v) fatty acid free BSA, pH 7.4 at 4 °C, will last 2 days in refrigerator			
Mitochondrial Oxygen Consumption			
Equipment			
Oxygraph Setup + Clark type oxygen electrode	Hansatech (PP Systems)	OXY1	
Thermoregulated Water Pump	ADInstruments	MLE2001	
Clark type Oxygen electrode	NA	NA	
Autopipette (1 mL)	Cole Parmer	SK-21600-70	Included with Oxy1
Small magnetic stir bar	Fisher Scientific	14-513-95	
Micropipette (10 µL)	Cole Parmer	SK-21600-60	
pH meter	VWR		
Chemicals			
KCl	Sigma - Aldrich	P9333-1KG	
Hepes	Sigma - Aldrich	H3375-500G	
KH ₂ PO ₄	Sigma - Aldrich	P5655-1KG	
MgCl ₂	Sigma - Aldrich	M1028-100ML	
EGTA	Sigma - Aldrich	E3889-100G	
Use to make mitochondrial oxygen consumption media: 120 mM KCL, 5 mM KH ₂ PO ₄ , 5 mM MgCl ₂ , 5 mM Hepes and 1 mM EGTA, pH 7.4 at 30 °C with 0.3% defatted BSA			
Rotenone (4 mM solution)	Sigma - Aldrich	R8875-5G	
Succinate (1 M solution)	Sigma - Aldrich	S3674-250G	
ADP (100 mM solution)	Sigma - Aldrich	A5285-1G	
Oligomycin (solution of 8 µg/mL in ethanol)	Sigma - Aldrich	75351	

FCCP	Sigma - Aldrich	C2920	
Mitochondrial Membrane Potential and Proton Motive Force			
Equipment			
TPMP electrode	World Precision Instruments.	DRIREF-2	
Chemicals-solutions do not need to be fresh but they do need to be kept in a freezer between runs			
Malonate (0.1 mM solution)	Sigma - Aldrich	M1296	
Oligomycin (8 µg/mL in ethanol), keep in freezer	Sigma - Aldrich	75351	
Nigericin (80 ng/mL in ethanol), keep in freezer	Sigma - Aldrich	N7143	
FCCP	Sigma - Aldrich	C3920	
TPMP	Sigma - Aldrich	T200	
TPMP solution: 10 mM TPMP, 120 mM KCL, 5 mM Hepes and 1 mM EGTA, pH 7.4 at 30 °C with 0.3% defatted BSA			