XF Palmitate-BSA FAO Substrate

Part #102720-100

Quickstart Guide

For use with XF^{e} and XF Extracellular Flux Analyzers

For Research Use Only

Seahorse Bioscience

A part of **Agilent Technologies**

PRODUCT DESCRIPTION

The XF Palmitate-BSA FAO (fatty acid oxidation) Substrate is a bio-available palmitate reagent for researchers studying substrate utilization who require a reliable source of conjugated fatty acids. The XF Palmitate-BSA FAO Substrate is manufactured at a 6:1 Palmitate:BSA ratio. Each lot is tested for activity and free fatty acid concentration [FFA].

CONTENTS

- 1 mM palmitate conjugated to 0.17 mM BSA in 150 mM NaCl, pH 7.2, 3 x 2 mL vials.
- 0.17 mM BSA control in 150 mM NaCl, pH 7.2, 3 x 2 mL vials. Lot-specific free fatty acid concentration [FFA] is listed on each box.

STORAGE CONDITIONS

Store at -20°C upon arrival. Aliquot and refreeze if desired. Glass vials are recommended for storage. The stability of the product is still under evaluation, but has a minimum of a 6 month shelf life when properly stored. Do not store in a frost-free freezer.

REAGENT PREPARATION

- Thaw solution at room temperature.
- Add undiluted substrate or control directly to the well prior to beginning the assay. We recommend adding the substrate directly to the microplate instead of to the injection ports to avoid inconsistent injection performance due to viscosity.

Recommended Volumes: Palmitate-BSA or BSA control	
XF^{e}24/XF24	87.5 µL
XF^{e}96/XF96	30 µL

INTRODUCTION

The ability to oxidize exogenously added fatty acids (FAs), as indicated by an increase in OCR, will be influenced by ATP demand, other substrates present in the assay medium (such as glucose), and stores of endogenous substrates (such as glycogen or triglycerides). Exogenously added fatty acids can also uncouple mitochondria, and cause an increase in the O_2 consumption rate not due to FAO.

Palmitate-BSA has been used extensively to examine free fatty acid oxidation. Addition of etomoxir to a cell and the subsequent return to the control rate of respiration demonstrate utilization of exogenous fatty acids (Ferrick, et. al 2008, Rowe et. al, 2013). A drop in OCR in both the palmitate and BSA control indicates that endogenous fatty acids were also being oxidized (Canto et al., 2009). Finally, if the OCR signal is insensitive to etomoxir or oligomycin, this indicates that the fatty acids are uncoupling the mitochondria (Divakaruni and Brand, 2011).

Because a change in OCR can be caused by utilization of exogenous FAs, endogenous FAs and/or uncoupling by FAs, Seahorse Bioscience developed the XF FAO Assay that can measure all three sources. With the combined use of the XF Palmitate-BSA FAO Substrate, etomoxir, and the XF Cell Mito Stress Test, one can drive cells to oxidize exogenous fatty acids while revealing the portion of the OCR signal generated by endogenous and exogenous fatty acid oxidation, as well as uncoupling.

The assay requires components that are not included with the XF Palmitate-BSA FAO Substrate. Please see following pages for a detailed description of how to perform the assay. This assay was developed using immortalized cell lines. Use of primary cultures may require optimization of reduced nutrient conditions.

Ferrick DA *et al.* (2008) Advances in measuring cellular bioenergetics using extracellular flux. Drug Discov Today. 13(5-6):268-74.

Rowe GC *et al.* (2013) Disconnecting mitochondrial content from respiratory chain capacity in PGC-1 deficient skeletal muscle. Cell Rep. 3(5): 1449-1456.

Canto C *et al.* (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458(23) 1056-1062.

Divakaruni AS and Brand MD (2011) The regulation and physiology of mitochondrial proton leak. Physiology 26:192-205.

Items Required But Not Provided

XF^{e}24/96 or XF24/96 Analyzer	www.seahorsebio.com
XF^{e}/XF FluxPak or FluxPak Mini	www.seahorsebio.com
Substrate-Limited Medium	See formulation below
FAO Assay Medium	See formulation below
Etomoxir (10 mM Stock Solution in H_2O)	Sigma-Aldrich, catalog #E1905, (+)-etomoxir sodium salt hydrate
XF Cell Mito Stress Test Kit	Seahorse Bioscience, catalog #103015-100

REAGENT PREPARATION

Substrate-Limited Medium

Component	Details
DMEM	Corning #17-207-CV or Life Technologies catalog #A14430-01 are DMEM formulations without glucose, glutamine, sodium pyruvate, or HEPES
0.5 mM Glucose	D-(+)-Glucose, catalog #G7021, Sigma-Aldrich
1.0 mM GlutaMAX™	GlutaMAX Supplement, catalog #35050061, Life Technologies
0.5 mM carnitine	L-Carnitine hydrochloride, Sigma-Aldrich, catalog #C0283
1% FBS	Fetal Bovine Serum

FAO Assay Medium

Component	1X concentration	5X concentration
NaCl	111 mM	555 mM
KCl	4.7 mM	23.5 mM
*$CaCl_2$	1.25mM	6.25mM
$MgSO_4$	2.0 mM	10 mM
NaH_2PO_4	1.2 mM	6 mM
Supplements	On the day of the assay, supplement 1X KHB with 2.5 mM glucose, 0.5 mM carnitine and 5 mM HEPES (final concentrations). Warm to 37°C and adjust pH to 7.4 with NaOH.	
Carnitine may be purchased as L-carnitine hydrochloride from Sigma. Make as a 0.5 M stock solution in H_2O and adjust pH to 7.2 with NaOH.		

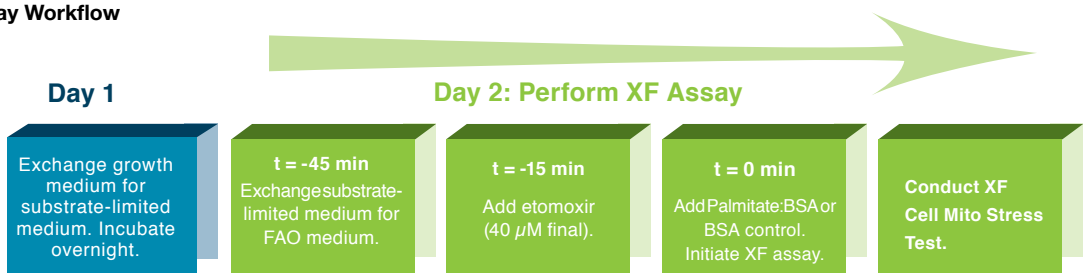
* This doucment was updated to include $CaCl_2$ in the FAO Assay Medium

FAO ASSAY

Note: The XF assay work flow deviates from standard XF assays, and includes two pre-treatment steps:

1) Selected groups are treated with etomoxir 15 minutes prior to the XF assay being initiated (t = -15 min)
2) Selected groups are provided BSA control or Palmitate:BSA prior to the XF assay being initiated (t = 0).

FAO Assay Workflow



RECOMMENDED VOLUMES

Recommended Volumes	XF^{e}/XF24	XF^{e}/XF96
Starting volume	375 µL	135 µL
400 µM Etomoxir (40 µM final)	37.5 µL	15 µL
BSA control or Palmitate:BSA	87.5 µL	30 µL
XF Cell Mito Stress Test Injections (all 10X) - final concentration		
Port A: 25 µg/mL Oligomycin (2.5 µg/mL final)	50 µL	20 µL
Port B: FCCP (TBD by user*)	55 µL	22 µL
Port C: 20 µM Rotenone/40 µM Antimycin A (2 and 4 µM final, respectively)	60 µL	24 µL

* Determination of maximal FCCP stimulation (maximal exogenous FA oxidation) should be conducted by performing an FCCP titration in the context of the FAO assay workflow (i.e. cells should be grown in substrate-limited medium, assayed in FAO assay media, and be FCCP titrated in the presence of BSA Control and Palmitate-BSA).

Determination of Fatty Acid Utilization in the XF^{e}/XF Analyzer

Figure A.

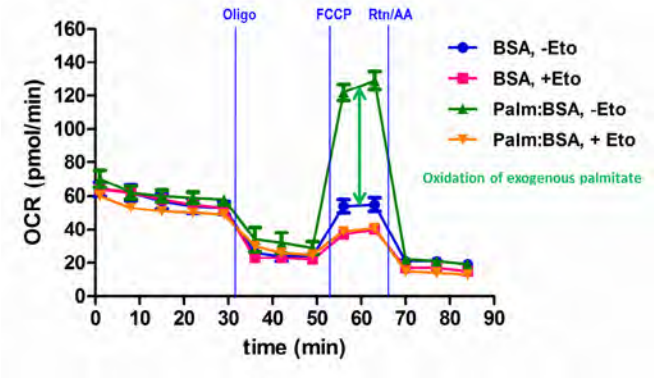


Figure A. Kinetic graph of the FAO/XF Cell Mito Stress Test assay. The green arrow indicates utilization of exogenous fatty acids. Note: in this case utilization of exogenous fatty acids was dependent on placing energetic stress (via FCCP) on the cells. This experiment illustrates that under conditions of substrate-limitation, and the combined use of the XF Palmitate-BSA FAO Substrate, etomoxir, and the XF Cell Mito Stress Test, one can determine the proportion of respiration that is supported by exogenous fatty acids.

Figure B.

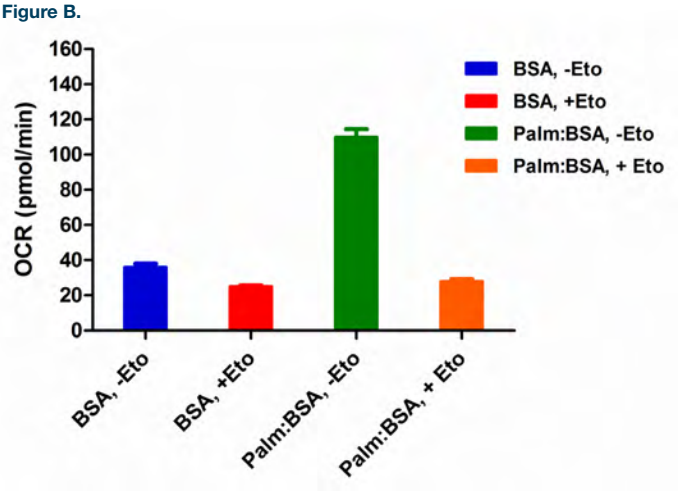


Figure B. Bar chart highlighting the differences in maximal respiration. Data taken from rate 10 measurement from Figure A. The use of the XF Cell Mito Stress Test can elucidate different metabolic parameters allowing the determination if the cells are oxidizing the exogeneous palmitate, as well as oxidizing endogenous fatty acids and/or causing uncoupling.

Download the MSDS for XF Palmitate-BSA FAO Substrate: http://www.seahorsebio.com/products/consumables/kits/FAO-substrate.php