O2k-Workshop on High Resolution Respirometry
IOC 162

Substrate-Uncoupler-Inhibitor-Titration (SUIT) protocols: Reference protocols 1 and 2

Alba Timón-Gómez, PhD, Mitochondrial Mage
Oroboros Instruments, Innsbruck, Austria
alba.timon@oroboros.at
High-resolution respirometry (HRR): NextGen-O2k

- Modular system for HRR for mitochondria and cell research
- Real-time OXPHOS analysis
- Living cells and diverse mitochondrial preparations

SUIT protocol:
Substrate-Uncoupler-Inhibitor Titration protocol
Coupling control

Mitochondrial pathways

SUIT reference protocol

Modified from Doerrier et al. 2018 Methods Mol Biol
SUIT reference protocol

Mitochondrial mapping in different models

Reference values

mt-database

mt-physiological diversity in different species, tissues, and cell types

Searching for specific defects/metabolic profiles in mitochondrial pathways in health and disease

Proficiency test
Fatty acid oxidation (F-pathway)

F-pathway: FA alone

Octanoylcarnitine ➔ Octanoylcarnitine

β-oxidation ➔ Acetyl-CoA

F-pathway: FA + Malate

Octanoylcarnitine ➔ Octanoylcarnitine

β-oxidation ➔ Acetyl-CoA

Malate

Oxaloacetate ➔ Citrate

Malate alone

without malic enzyme

Fumarate ➔ 2-Oxoglutarate

Citrate ➔ Succinate

Malate

P

F

2-Oxoglutarate

Malate

P

F

Succinate

2-Oxoglutarate

with malic enzyme

Fumarate ➔ 2-Oxoglutarate

Citrate ➔ Succinate

Malate

P

F

2-Oxoglutarate

Malate

P

F

Succinate

2-Oxoglutarate

Timón-Gómez et al., unpublished data
Malate anaplerotic pathways

Timón-Gómez et al., unpublished data
Fatty acid oxidation

SUIT-RP2

Timón-Gómez et al., unpublished data
Cytochrome c control efficiency

\[ J_{cyt\ c} = \frac{(J_{CHNOc} - J_{C_HNO})}{J_{CHNOc}} \]

Timón-Gómez et al., unpublished data
Modifications of SUIT-RP for PBMCs and platelets

SUIT-001 ce O2 D004
Modifications of SUIT-RP for blood cells

**a**

![Graph a](image)

**b**

![Graph b](image)

Timón-Gómez et al., unpublished data
SUIT reference protocol for database generation

imt, thom, pfi

ce-pce

Modified from Doerrier et al. 2018 Methods Mol Biol
Thank you!

Alba Timón-Gómez, PhD, *Mitochondrial Mage*
Oroboros Instruments, Innsbruck, Austria

alba.timon@oroboros.at