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**SUIT reference protocol: RP2-Pfi**(pre03)

**O2k high-resolution respirometry**

RP2: FAO-substrate control 2016-01-20

D + mt + Oct + Mtit + P + c + (NADH) + G + S + U + Gp + Rot + Ama + AsTm + Azd

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | |  | | --- | | FAO | | CI&FAO | CI&FAO | CI&II&FAO | CI&II&FAO&GpDH | CII&GpDH | CIV | | |
| *E* |  |  |  |  | **PGMSOct** | **PGMSOctGp** | **SGp** |  | **AsTm** |  |
| *P* |  | **OctM** | **PMOct** | **PGMOct** | **PGMSOct** |  |  |  | | |
| *L* |  |  |  |  |  |  |  |  | | |
|  | D | Oct+M | P | G | S | Gp | Rot | Ama+AsTm+Azd | | |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **DatLab file:** 2016-  **Experimental code:**  **Operator:** | | | | | **O2k:** | **P**\_\_ | |
|  | Chamber | |
| **Event** | **Mark name** | **Stock**  **[mM]** | **Final conc. in O2k 2 ml** | **Comment** | **Titration**  **[µl]** | **A** | **B** |
| **MiR** |  |  | MiR05+CtlCr |  |  |  |  |
| **O2** |  |  | ~450 µM |  |  |  |  |
| **D** |  | 500 | 7.5 mM |  | 30 |  |  |
| **Pfi** |  |  |  |  |  |  |  |
| **O2** | ROX |  | ~450 µM |  |  |  |  |
| **Oct** | Oct(P) | 100 | 0.5 mM |  | 10 |  |  |
| **M.05** | OctM.05(P) | 50 | 0.05 mM |  | 2 |  |  |
| **M.1** | OctM.1(P) | 50 | 0.1 mM |  | 2 |  |  |
| **M2** | OctM2(P) | 400 | 2 mM |  | 9.5 |  |  |
| **P** | PMOct(P) | 2000 | 5 mM |  | 5 |  |  |
| **c** | PMOctc(P) | 4 | 10 µM |  | 5 |  |  |
| **NADH** | PMOctcNADH(P) | 280 | 2.8 mM | only if *FCFc* >0.1 | 20 |  |  |
| **G** | PGMOct(P) | 2000 | 10 mM |  | 10 |  |  |
| **S** | PGMSOct(P) | 1000 | 50 mM |  | 100 |  |  |
| **U** | PGMSOct(E) | 1  CCCP | 0.5 – 5 µM |  | 1 µl steps |  |  |
| **Gp** | PGMSOctGp(E) | 1000 | 10 mM |  | 20 |  |  |
| **Rot** | SGp(E) | 1 | 0.5 µM |  | 1 |  |  |
| **Ama** | ROX | 5 | 2.5 µM |  | 1 |  |  |
| **O2** |  |  | ~450 µM |  |  |  |  |
| **As** |  | 800 | 2 mM |  | 5 |  |  |
| **Tm** | CIV(E) | 200 | 0.5 mM | ~20 min | 5 |  |  |
| **Azd** | ROX | 4000 | ≥100 mM | ~10 min | 100 |  |  |
| **O2** | ROX |  | ~450 µM | 400 -> 250 µM |  |  |  |

**Sample (Pfi):**