Mitochondrial production of hydrogen peroxide

# Reagents

**- Respirometry buffer**  aliquot of 50 mL at -20°C

- **DTPA (acronyme)** aliquot of 30µL at -20°C

- **Amplex Red** (10-Acetyl-3,7-dihydroxyphenoxazine) -alicot of 20 µL in opaque tube at -20 °C.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Cayman ref | Store at | Final conc. | FW | DMSO |
| Amplex red | 10010469-5mg | -20°C | 6,67 mM | 257.2 | 2,9 mL |

-**Horse Radish Peroxidase** (HRP) - alicot of 20 µL in opaque tube at -20 °C.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Sigma ref | Store at | Final conc. kU mL | Approx kUnits mg | 1X MAS |
| HRP | P6782-5mg 5KU | 4°C | 5 | 1 | 1 mL |

HRP : 5000kU dilué dans 1mL,dilution au 10ème dans 10 aliquots de 1mL (500U mL), aliquot de 30µL sur 1mL et 9 autres aliquots de 1mL congelés au -20°C.

HRP 10 mM (Cf = 500 U mL)

Amplex Red (Cf = 6,67 µM)

- **Hydrogen peroxide** (H2O2) - 17.6 M (50 wt. % in H2O, stabilized) Sigma 516813

Freshly prepared by serial dilution and keep in dark at 4°C

17.6 10-2M = 10µL H2O2 17.6M in 990 µLH2O δ

17.6 10-4M = 10µL H2O2 17.6 10-2M in 990 µLH2O δ

35.2 10-6M = 20µL H2O2 17.6 10-4M in 990 µLH2O δ

# Methods:

-2 200 µL red **muscle** homogenate (1mg ml-1)

*Or*

-2 200 µL **liver** homogenate (5mg ml-1)

*-Add 6µL DTPA (*Cf = 15µM) with a pipette

-Add 8µL of Ap5A 25mM (Cf = 100µM) with a pipette

*----------------Close the chambers and turn off the light-----------------*

*----------------------Turn on the fluorescents----------------------*

-Add 3 µL Amplex Red (Cf= 10µM)

-Add 4 µL HRP (Cf= 1 U mL)

-----------------------------------Reading for 2 sec----------------------------------

-Add 5 µL **H2O2** 35.2 µM (mol nb = 88 nmol).

-----------------------------------Reading for 2 sec----------------------------------

-Add 5 µL **H2O2** 35.2 µM (mol nb = 88 nmol).

-----------------------------------Reading for 2 sec----------------------------------

-Add 5µL of **Pyruvate** 2M (Cf = 5mM)

-Add 2.5µL of **Malate** 400mM (Cf = 0.5mM)

-----------------------------------Reading for 2 minutes----------------------------------

-Add 20µL of **Succinate** 1M (Cf = 10mM)

-----------------------------Reading for 5 minutes----------------------------------

-Add 5 µL **H2O2** 35.2 µM (mol nb = 88 nmol).

-----------------------------------Reading for 2 sec----------------------------------

-Add 5 µL **H2O2** 35.2 µM (mol nb = 88 nmol).

-----------------------------------Reading for 2 sec----------------------------------

-Add 16µL of **ADP** 250mM (Cf = 2mM)

-----------------------Reading for Liver: **15** minutes, Red Muscle: **5** minutes----------------

-Add 5 µL **H2O2** 35.2 µM (mol nb = 88 nmol).

-----------------------------------Reading for 2 secondes----------------------------------

-Add 8µL of **cATR** 1mM (Cf = 4μM)

-----------------------Reading for Liver: **5** minutes, Red Muscle: 1**5** minutes----------------

-Add 5 µL **H2O2** 35.2 µM (mol nb = 88 nmol).

-----------------------------------Reading for 2 secondes----------------------------------

-Add 1µL of **Antimycin A** 5mM (Cf = 2.5µM)

*-------------------------------Reading 15 min -----------------------------*

**Cleaning:**

* *3 times distilled water*
* *2 times Eth 70%*
* *5 min Eth 70%*
* *10 min Eth 100%*
* *5 min Eth 100% ----- or: Overnight Eth 70%*
* *2 times Eth 70%*
* *3 times distilled water*