Mitochondrial Respiration Reagent Kit

Item No. 502898

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GENERAL INFORMATION

Materials Supplied

Item No.	Item Name	Quantity/Size	Storage
11342	Oligomycin A	1 vial/1 mg	-20°C
15218	FCCP	1 vial/1 mg	-20°C
17811	BAM15	1 vial/1 mg	-20°C
34799	Antimycin A Complex	1 vial/1 mg	-20°C
13995	Rotenone	1 vial/1 mg	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Ethanol, 200 proof

INTRODUCTION

Description

The components of this kit enable controlled modulation of mitochondrial function across the electron transport chain (ETC) in cultured cells. The reagents can be used for mitochondrial stress testing, bioenergetic profiling, and mechanistic studies of oxidative phosphorylation.[1] Each component in this kit is provided separately, and instructions are included to reconstitute these reagents for direct dilution into cell culture medium. Some reagents may be provided in excess and may be properly disposed of after cell medium preparation. Prior to use, reagents must be stored as indicated on page 3.

Oligomycin A is an inhibitor of mitochondrial complex V, also known as F_1F_o-ATP synthase.[2,3] It can be used to quantify ATP-linked oxygen consumption.

FCCP is a mitochondrial uncoupler that collapses the mitochondrial proton gradient, causing the ETC to operate at maximal capacity.[4,5] FCCP can be used to determine respiratory capacity.

BAM15 is a next-generation mitochondrial uncoupler.[6] Compared with FCCP, BAM15 does not depolarize the plasma membrane and exhibits reduced cytotoxicity and off-target effects, making it useful for longer or more sustained studies of mitochondrial function.

Antimycin A complex is a bacterial metabolite complex that contains several antimycins, including antimycin A_3, which is an inhibitor of mitochondrial complex III, also known as cytochrome bc_1 complex.[7,8] High concentrations of antimycin A complex, in combination with rotenone, can be used to fully inhibit mitochondrial respiration.

Rotenone is an inhibitor of mitochondrial complex I, also known as NADH dehydrogenase.[9] It can be used together with antimycin A complex to determine non-mitochondrial oxygen consumption.[10]

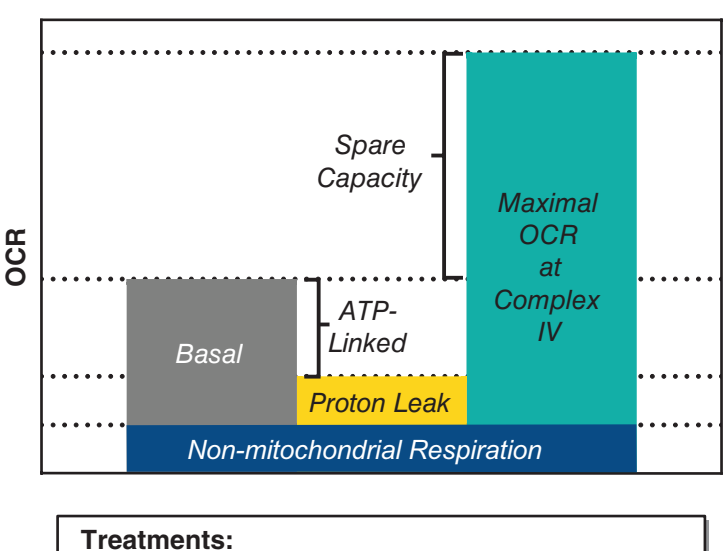

Figure 1. Determination of mitochondrial respiration parameters. Basal Oxygen Consumption Rate (OCR) is measured in the presence of vehicle. ATP-linked respiration is calculated as the difference between vehicle and oligomycin A-treated OCR, while maximal OCR is determined following mitochondrial uncoupling with BAM15 or FCCP. Spare respiratory capacity is calculated as maximal OCR minus basal OCR. Non-mitochondrial respiration is determined after inhibition with a combination of antimycin A and rotenone and represents residual oxygen consumption independent of the mitochondrial ETC.

PREPARATION

Reagent Preparation

General Guidelines

- Concentrated stock solutions may be stored at or below -20°C, protected from light for up to six months. Prepare single-use aliquots and store on ice during handling to minimize volume loss from evaporation.
- Intermediate stock solutions in cell culture medium should be prepared just prior to use and discarded within a day. *NOTE: When preparing intermediate stock solutions, it is not necessary to dilute the entire concentrated stock solution all at once; generate only the volume needed for upcoming experiments.*
- Final concentrations of solvents in cell culture medium should be kept at ≤0.1% (v/v).
- Recommended final concentrations are provided as general guidelines. Actual performance may depend on cell type, density, medium formulation, and experimental design. Users should perform an optimization experiment to determine the appropriate concentration for each reagent.

Preparation of Individual Stock Solutions

1. **Oligomycin A - (Item No. 11342)**

   Prepare a 5 mM concentrated stock solution of oligomycin A by adding 253 µl of ethanol to the vial and mix until fully dissolved. Immediately prior to use, prepare a 50 µM intermediate stock solution by diluting the 5 mM concentrated stock solution 100-fold in cell culture medium. Dilute the 50 µM oligomycin A solution into cell culture medium to achieve the final desired working concentration, which typically ranges from 0.1-2.5 µM.

2. **FCCP - (Item No. 15218)**

   Prepare a 10 mM concentrated stock solution of FCCP by adding 393 µl of ethanol to the vial and mix until fully dissolved. Immediately prior to use, prepare a 100 µM intermediate stock solution by diluting the 10 mM concentrated stock solution 100-fold in cell culture medium. Dilute this 100 µM FCCP solution into cell culture medium to achieve the final desired working concentration, which typically ranges from 0.125-5 µM.

NOTE: FCCP needs to be titrated carefully for each cell type, and the optimal concentration may change with seeding density and culture conditions. Minimize exposure to light and store in amber vials.

3. **BAM15 - (Item No. 17811)**

   Prepare a 10 mM concentrated stock solution of BAM15 by adding 294 µl of ethanol to the vial and mix until fully dissolved. Immediately prior to use, prepare a 100 µM intermediate stock solution by diluting the 10 mM concentrated stock solution 100-fold in cell culture medium. Dilute this 100 µM BAM15 solution into cell culture medium to achieve the final desired working concentration, which typically ranges from 0.3-5 µM.

NOTE: BAM15 needs to be titrated carefully for each cell type, and the optimal concentration may change with seeding density and culture conditions. Minimize exposure to light and store in amber vials.

4. **Antimycin A Complex - (Item No. 34799)**

   Prepare a 10 mM concentrated stock solution of antimycin A complex by adding 182 µl of ethanol to the vial and mix until fully dissolved. Immediately prior to use, prepare a 100 µM intermediate stock solution by diluting the 10 mM concentrated stock solution 100-fold in cell culture medium. Dilute this 100 µM antimycin A complex solution into cell culture medium. For mitochondrial respiration studies, concentrations ranging from 0.5-2 µM are recommended.

NOTE: Minimize exposure to light and store in amber vials.

4. **Rotenone - (Item No. 13995)**

   Prepare a 10 mM concentrated stock solution of rotenone by adding 254 µl of ethanol to the vial and mix until fully dissolved. Immediately prior to use, prepare a 100 µM intermediate stock solution by diluting the 10 mM concentrated stock solution 100-fold in cell culture medium. Dilute this 100 µM rotenone solution into cell culture medium to achieve the final desired working concentration, which typically ranges from 0.01-1 µM.

References

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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.