Technical Manual

Fatty Acid Oxidation (FAO) Assay Kit

- **Catalogue Code: BR00001**
- **Research Use Only**

Please note:

The FAO Assay kit is shipped in multiple separate components (in 6 vials) to ensure reagent integrity for at least one week in the absence of dry ice. Store the kit at -20℃ prior to reconstitution. Please follow instructions below to reconstitute FAO Assay Solution and 20x FAO Substrate Solution.

Reconstitution of one FAO Assay Kit

(5 ml FAO Assay Solution and 0.25 ml 20x FAO Substrate)

Reconstitution of FAO Assay Solution (5 ml):

1. Thaw Reagent 1 (0.5 ml in brown microtube) at room temperature. Keep FAO Assay Solution (5 ml in green cap vial) on ice.

2. Transfer Reagent 1 solution to FAO Assay Solution and immediately mix solution to prevent reagent precipitation. Keep the mixed solution on ice shielded from light.

3. Tap Reagent 2 vial (clear microtube) to deposit contents inside. Transfer Reagent 2 contents to FAO Assay Solution and dissolve contents by gentle agitation (rinse the clear vial with some FAO Assay Solution to ensure complete transfer if necessary). Gently agitate FAO Assay Solution on ice shielded from light for ~5 min. The reconstituted FAO Assay Solution should be stored at -70℃.

Reconstitution of 20x FAO Substrate (0.25 ml):

1. Tap 20x FAO Substrate vial (clear microtube) to deposit contents inside.

2. Add 0.25 ml ddH_2O (in clear microtube) to 20x FAO Substrate vial and vortex tube to dissolve content. The reconstituted 20x FAO Substrate solution should be stored at -70℃.

Fatty Acid Oxidation (FAO) Assay Kit (Cat #BR00001)

COMPONENTS:

- FAO Assay Solution- 5 ml (for 100 wells), store at -70°C (shield solution from light during assay)
- 20x FAO Substrate (20x Octanoyl-CoA) - 0.25 ml, store at -70°C
- 10x Cell Lysis Solution- 25 ml, store at 4°C

PRODUCT DESCRIPTION:

The fatty acid β-oxidation activity assay is based on the oxidation of octanoyl-CoA, which is coupled to NADH dependent reduction of INT to INT-formazan. The formazan product exhibits an absorption maximum at 492 nm (extinction coefficient = 18 mM-1cm-1), allowing for sensitive measurement of FAO activity present in cell/tissue extracts. Note that FAO activity is influenced by the mitochondrial contents of the cell/tissue used. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (10-20 mg tissue in 0.5 ml).

2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.

3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 – 2 mg/ml. Always Keep lysates on ice during assay.

Reagent thawing:

Keep thawed FAO Assay Solution and 20x FAO Substrate on ice shielded from light. Do not over thaw. Gently agitate solution prior to pipetting. It is important to minimize the time the reagents are thawed. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 19 parts of FAO Assay Solution, e.g. 25 µl dH_2O mixed with 475 µl FAO Assay Solution. Keep the solution on ice.

Reaction solution is prepared by mixing 1 part of 20x FAO Substrate and 19 parts of FAO Assay Solution, e.g. 25 µl 20x FAO Substrate mixed with 475 µl FAO Assay Solution. Keep solution on ice and use immediately.

Enzyme assay:

1. Thaw the lysates quickly and keep on ice (do not over thaw). Each sample is treated with 50 µl control solution and 50 µl reaction solution. Add 20 µl of each sample to a plain (uncoated) 96-well plate placed on ice ***in duplicate.***

   Note: For drug discovery application, add 1 µl of a drug inhibitor to both control and reaction wells and mix by pipetting up and down.

2. After all samples have been pipetted to the plate in duplicate, swiftly add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 30 - 60 min (do not use CO_2 incubator). Cherry red color should gradually appear in wells.

3. Measure $O.D._{492}$ nm using a plate reader at 30 min and at 60 min.

4. Subtract control well reading from reaction well reading for each sample for each time point. Use the subtracted reading (ΔO.D.) for enzyme activity calculation. If incubation for 30 min, sample FAO activity in IU/L = µmol/(L•min) = ΔO.D. × 1000 × 70µl / (30 min × 0.5 cm × 18 × 20µl) = ΔO.D. × 12.96. If incubation for 60 min, FAO activity = ΔO.D. x 6.48. Enzyme activity can be presented as units/µg proteins.

   Note: Incubation time may be increased to 120 min to increase ΔO.D. for samples exhibiting low FAO activity (FAO activity = ΔO.D. X 4.05).

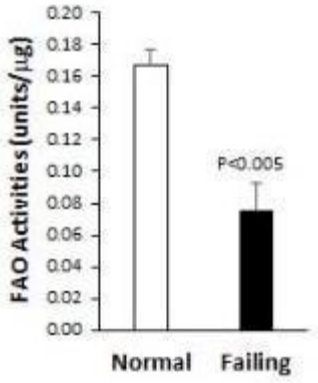
   Note: In the activity equation above, the 0.5cm is based on U-shaped wells, if using flat bottomed plate adjust the well height accordingly.

5. If you would like to run a Positive Controls cell with a high mitochondrial content should be used e.g. heart or muscle tissue extract (not included).

Additional information:

The assay solution contains DMSO and iodonitrotetrazolium violet.
Please refer to the product page of our website or contact us for MSDS information.

Data



Tissue homogenates were prepared from normal hamster heart (F1B strain) and failing heart (TO2 strain). FAO activities were assayed by the FAO Assay kit, showing that the failing hamster heart exhibits reduced capacity for fatty acid oxidation.

Notes

Notes

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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