BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Aldehyde Oxidase (AO) Assay Kit (Cat #: E-157)

COMPONENTS: AO Reaction: 0.5 ml (for 50 samples), store at 4°C
AO Control: 0.5 ml, store at 4°C
Detection Reagent: 15 g, store at 4°C
10x Tissue Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The AO enzyme activity assay kit uses glutaraldehyde as substrate and generates H_2O_2. Potassium iodide (KI) is used for colorimetric detection of hydrogen peroxide at 405 nm (molar extinction coefficient 5.3 $mM^{-1}cm^{-1}$). Each kit is sufficient for ~50 samples using a 96-well format. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue lysates:

1. Prepare 1x Tissue Lysis Solution by diluting 10x Tissue Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Tissue Lysis Solution by pipetting up and down gently. Leave homogenate on ice for 5 min with agitation. If homogenate is overly turbid, add more 1x Tissue Lysis Solution and repeat homogenization. Tissue should be mechanically homogenized in ice-cold 1x Tissue Lysis Solution (5 - 10 mg tissue in 0.5 ml).
2. Centrifuge homogenate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant (lysate) is harvested and should be stored at -80ºC after use.
3. Use the BCA protein assay method to determine lysate protein concentration. Dilute lysate protein concentration to 1 − 5 mg/ml using ice-cold 1x Tissue Lysis Solution. *Normalization of lysate protein concentration is recommended.*

Preparation of detection solution:

Each reaction/control well requires 0.1 ml freshly prepared detection solution, i.e., 0.2 ml per sample. Calculate the amount of detection solution required for each assay. Add 1.4 g of Detection Reagent to each ml of dH_2O (scale up as needed). Vortex solution vigorously to completely dissolve reagent. Keep solution at room temperature shielded from light. Discard unused solution after each assay.

Enzyme assay:

1. Use a plain (uncoated) 96-well plate for the assay. Add 10 µl 1x Tissue Lysis Solution to duplicate wells (blank wells). Add 10 µl of each sample to duplicate wells (sample wells). Duplicate wells are for control and reaction.
2. Add 10 µl AO Control to each control well. Add 10 µl AO Reaction to each reaction well.
3. Mix contents by gentle agitation of plate for 10 sec. Cover plate and shield from light. Incubate in a 37°C incubator for 20 min (do not use CO_2 incubator).
4. Remove plate from incubator. Keep plate at room temperature shielded from light for 10 min. Proceed to prepare detection solution (see *Preparation of detection solution* above) during the 10-min incubation.
5. Add 0.1 ml freshly prepared detection solution to each control well and reaction well. Mix contents by gentle agitation for 10 sec. Keep plate at room temperature shielded from light for 3 min.
6. Measure $O.D._{405\ nm}$ using a plate reader.
7. Subtract control well reading from reaction well reading to generate ΔO.D. for blank (**$\Delta O.D._{Blank}$**) and each sample (**$\Delta O.D._{Sample}$**). Then subtract $\Delta O.D._{Blank}$ from $\Delta O.D._{Sample}$ to generate **$\Delta\Delta O.D._{Sample}$** for each sample.

   Enzyme activity in IU/L = µmol/(L•min) = **$\Delta\Delta O.D._{Sample}$** × 1000 × 120 µl / (30 min × 0.6 cm × 5.3 × 10 µl) = **$\Delta\Delta O.D._{Sample}$ × 125.79**.

Additional information:

- Detection reagent contains potassium iodide. AO Reaction contains a low concentration of glutaraldehyde. Please refer to the product webpage or contact us for SDS information of potassium iodide and glutaraldehyde.