BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Monoamine Oxidase (MAO) Assay Kit (Cat #: E-155)

COMPONENTS: MAO Substrate: 15 mg, store at 4°C
MAO Control: 4 ml, store at -20°C (**contains sodium azide; avoid skin contact**)
Detection Reagent: 15 g (50 samples), store at 4°C
10x Tissue Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The MAO assay kit uses dopamine as substrate and detects the activities of both MAO-A and MAO-B present in cell/tissue lysates. Potassium iodide (KI) is used for colorimetric detection of hydrogen peroxide at 405 nm (molar extinction coefficient 5.3 $mM^{-1}cm^{-1}$). 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. 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 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 stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. Normalize sample protein concentration to 1 − 5 mg/ml using ice-cold 1x Tissue Lysis Solution.

Preparation of MAO Reaction solution:

Thaw MAO Control solution. *Do not over thaw.* Keep solution on ice shielded from light.

Prepare a fresh MAO Reaction solution by adding 1.5 mg MAO Substrate to 0.5 ml MAO Control solution. Briefly vortex solution to dissolve substrate. Keep solution on ice and shielded from light. Use immediately.

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.

Enzyme assay:

1. Use a plain (uncoated) 96-well plate for the assay. Add 10 µl MAO Control to each well of the control set. Add 10 µl freshly prepared MAO Reaction to each well of the reaction set.
2. Add 10 µl 1x Tissue Lysis Solution to a control well and reaction well (blank wells). Then add 10 µl of each sample to a designated control well and reaction well (sample wells).
3. Mix contents by gentle agitation for 10 sec. Cover plate (shielded from light) and 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 protocol above).
5. Add 0.1 ml freshly prepared detection solution to each control well and reaction well. Mix contents by gentle agitation. Keep plate at room temperature shielded from light for 3 min.
6. Measure $O.D._{405\ nm}$ using a plate reader. Subtract control well reading from reaction well reading to generate ΔO.D. for blank (**$\Delta O.D._{Blank}$**) and each sample (**$\Delta O.D._{Sample}$**).
7. 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:

MAO Control solution contains sodium azide. Detection Reagent contains potassium iodide. Please refer to the product webpage or contact us for SDS information.