ab241031 Monoamine Oxidase (MAO) Assay Kit (Fluorometric)

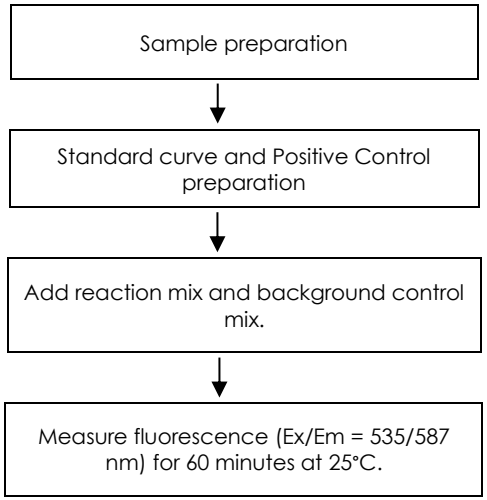
For the measurement of total MAO, MAO-A, and MAO-B activity in various biological samples.
For research use only - not intended for diagnostic use.

Background:

Monoamine Oxidase (MAO) Assay Kit (ab241031) is a sensitive assay to detect total monoamine oxidase activity as well as MAO-A and MAO-B isoenzyme activities separately in the presence of Clorgyline and Selegiline - specific inhibitors for MAO-A and MAOB, respectively. The assay is based on the fluorometric detection of H_2O_2, one of the by-products generated during the oxidative deamination of the MAO substrate (Tyramine). The assay can detect as little as 5 µU of MAO enzymatic activity.

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Solubilize lyophilized components, thaw OxiRed™ Probe, H_2O_2 Standard and Assay Buffer 18 (aliquot if necessary); get equipment ready.
- Prepare standard curve and samples in duplicate.
- Add buffer, MAO-B Inhibitor or MAO-A Inhibitors to samples to measure total MAO, MAO-A, or MAO-B activity, respectively.
- Add samples and MAO-A Positive Control to MAO Reaction Mix. Add background control samples to Background Reaction Mix.
- immediately measure fluorescence at Ex/Em= 535/587 nm.

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 18	25 mL	-20°C	-20°C
MAO Substrate	1 vial	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
H_2O_2 Standard	100 µL	-20°C	-20°C
Developer Solution V	1 Vial	-20°C	-20°C
MAO-A Positive Control	1 Vial	-20°C	**-80°C**
MAO-A Inhibitor	1 vial	-20°C	-20°C
MAO-B Inhibitor	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 18 was previously labelled as Assay Buffer XVIII, and OxiRed™ Probe as OxiRed Probe and Ethanol Probe (in DMSO, anhydrous). The composition has not changed.

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm.
- 96 well black plate with flat bottom.
- Dounce homogenizer (if using tissues or cells)
- MilliQ water or other type of double distilled/deionized water (ddH2O)
- For tissue samples, we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Assay Buffer 18 and **H_2O_2 Standard** are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C and protect from light and moisture.

OxiRed™ Probe: Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.
For multiple use aliquot and store at −20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).

MAO Substrate: Reconstitute with 110 µL ddH_2O to generate a 100 mM solution. Keep on ice during the assay. Use within two months.

Developer Solution V: Reconstitute with 220 µL of Assay Buffer and mix well. Use within two months.

MAO-A Inhibitor: Reconstitute with 250 µL ddH_2O to generate a stock solution of 2 mM. Make a 10 µM working solution by adding 5 µL of the 2 mM stock solution into 995 µL of ddH_2O. Store the stock solution at -20°C. Inhibitor working solution can be stored at 4°C to use within 24 hours.

MAO-B Inhibitor: Reconstitute with 250 µL ddH_2O to generate a stock solution of 2 mM. Make a 10 µM working solution by adding 5 µL of the 2 mM stock solution into 995 µL of ddH_2O. Store the stock solution at -20°C. Inhibitor working solution can be stored at 4°C to use within 24 hours.

MAO-A Positive Control: Reconstitute with 40 µL of Assay Buffer 18. Store at -80°C.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare serial dilution of H_2O_2 Standard as follows:

1. Dilute H_2O_2 Standard to 10 mM by adding 10 µL of 0.88 M H_2O_2 Standard to 870 µL ddH_2O. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.
2. Further dilute to 0.1 mM by adding 10 µL of 10 mM H_2O_2 Standard into 990 µL of ddH_2O. **Use this to prepare standard curve.**
3. Add 0, 2, 4, 6, 8, and 10 µL of 0.1 mM H_2O_2 Standard into a series of wells in a 96-well plate to generate a 0, 200, 400, 600, 800, and 1000 pmol/well H_2O_2 Standard. Adjust the volume to 50 µL/well with Assay Buffer 18.

Standard #	Volume of 0.1 mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	H_2O_2 in well (pmol/well)
1	0	50	50	0
2	2	48	50	200
3	4	46	50	400
4	6	44	50	600
5	8	42	50	800
6	10	40	50	1000

ΔNote: Dilute H_2O_2 Standard just before use. Diluted H_2O_2 is unstable.

Sample Preparation:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. We recommend that you use fresh samples for the most reproducible assay.

For tissue samples we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.

Biological fluids (e.g. serum, plasma):
1. Biological fluids may be assayed directly.

Mammalian tissues and cell lines:
1. Homogenize tissue (1-10 mg) / cells (1×10^6 - 1×10^7) (using Assay Buffer 18 (0.1 mg/µL).
2. Centrifuge the homogenate (10,000 X g for 10 minutes at 4°C).
3. Collect supernatant and keep on ice while in use.

Assay Procedure:

- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate. Make sure to plan the number of wells around if you are measuring total activity, MAO-A or MAO-B activity.

Positive Control:

1. For Positive Control(s), add 4 µL of MAO-A Positive Control solution into desired well(s) and adjust the total volume to 50 µL/well with Assay Buffer 18.

Samples:

1. To measure <u>total MAO</u> activity, add 1-40 µL of supernatant into desired well(s) of a 96-well plate and adjust total volume to 50 µL/well with Assay Buffer 18.
2. To measure <u>MAO-A Activity</u>, add 1-40 µL of supernatant and 10 µL of 10 µM Selegiline working solution. Adjust the total volume to 50 µL/well with Assay Buffer 18.
3. To measure <u>MAO-B</u> activity, add 1-40 µL of supernatant and 10 µL of 10 µM Clorgyline working solution. Adjust the total volume to 50 µL/well with Assay Buffer 18.

ΔNote: For unknown samples, we suggest doing a pilot experiment & testing several dilutions to ensure the readings are within the Standard Curve linear range.

ΔNote: For samples having H_2O_2 background, prepare parallel sample well(s) as background control.

4. Incubate the plate for 10 minutes at 25°C.

ΔNote: This incubation step is required for efficient inhibition of the MAO-A/MAO-B in the positive control or samples.

Reaction Mix:
Prepare 50 µL of MAO Reaction Mix and Background Mix for each reaction. Prepare a bulk mix to ensure consistency.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 18	47	48
Developer Solution V	1	1
MAO Substrate	1	-
OxiRed™ Probe	1	1

1. Mix bulk Reaction Mix by inversion. Add 50 µL of the bulk Reaction Mix to each standard, sample, and positive control well.
2. Add 50 µL of Background Reaction Mix into the background control sample wells.

Measurement:
1. Immediately measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode at 25°C for 60 minutes.

Calculations:

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract 0 Standard reading from all readings.
3. Take the end-point readings of the standards and plot the corrected values for each standard as a function of the final concentration of H_2O_2.
4. If sample background control reading is high, subtract the sample background control reading from sample readings.
5. Plot the RFU versus time curve for each sample. Take 2 time points T1 and T2 within the linear range of the RFU versus time curves. Calculate Total MAO, MAO-A and MAO-B activity of the test samples: ΔRFU = RFU2 -RFU1 (RFU1 at T1 and RFU2 at T2).
6. Apply the ΔRFU to the H2O2 Standard Curve to get B pmol of H_2O_2 generated by MAOs during the reaction time (ΔT = T2- T1).
7. Calculate total MOA(MAO-T)/MAO-A/MAO-B activity by using the following equation:

$$Sample\ MAO\ activity\ (A) = \frac{B}{(\Delta T * V)} * D$$

Where:
A = Total MAO/MAO-A/MAO-B activity in pmol/minute/mL = µU/mL.
B = Amount of H_2O_2 in sample well calculated from standard curve in pole.
V = Sample volume added in the sample wells (mL).
D = Sample dilution factor if sample is diluted to fit within the standard curve range.

Unit Definition: One unit of MAO activity is the amount of enzyme that generates 1.0 µmol of H_2O_2 per minute at 25°C

MAO specific activity can also be expressed as µU/mg of protein.

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:

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