ab284511 – Monoamine Oxidase B (MOA-B) Inhibitor Screening Kit (Fluorometric)

For the screening of potential MOA-B inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284511

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer 18	25 mL	-20°C
MAO Substrate	1 vial	-20°C
MAO-B Enzyme	1 vial	-20°C
MAO-B Inhibitor	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Developer Solution V	1 vial	-20°C

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

Materials Required, Not Supplied

- 96-well black plate with flat bottom
- Multi-well spectrophotometer

Reagent Preparation

– Before using the kit, spin the tubes prior to opening.

Assay Buffer 18: Bring to room temperature before use. Store at -20°C.

OxiRed™ Probe: Bring to room temperature before use. Protect from light & moisture. Store at -20°C. Stable for two months.

MAO-B Enzyme: Reconstitute with 25 µL Assay Buffer 18. Mix well. Aliquot & store at -80°C. Stable for two months.

MAO Substrate: Reconstitute with 110 µL ddH_2O. Store at -20°C. Stable for two months.

Developer Solution V: Reconstitute with 220 µL Assay Buffer 18. Mix well. Store at -20°C. Stable for two months.

MAO-B Inhibitor: Reconstitute with 250 µL ddH_2O to make a stock solution of 2 mM. Mix well. Make a 10 µM working solution by adding 5 µL of the 2 mM stock solution into 995 µL ddH_2O. Store the stock solution at -20°C. Stable for two months. Inhibitor's working solution can be stored at 4°C to use within 24 hrs.

Assay Protocol

Version 2b, Last updated June 26, 2025

Screening Compounds, Inhibitor Control, and Blank Control Preparations

1. Dissolve candidate test compounds into proper solvent.
2. Dilute to 10X concentration with Assay Buffer 18.
3. Add 10 µL diluted test compounds (S), working solution of Inhibitor Control (IC) and Assay Buffer 18 (Enzyme Control; EC) into assigned wells.

Δ Note: *Preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2% include a Solvent Control to test the effect of the solvent on enzyme activity.*

Δ Note: *Optional: To check the possible inhibitory effect of test inhibitors on Developer Solution V, prepare a parallel test inhibitor well (TI). MAO-B Inhibitor does not inhibit the Developer Solution V.*

MAO-B Enzyme Solution preparation:

1. Dilute the Enzyme stock solution 5 times by adding 2 µL of MAO-B Enzyme Stock Solution into 8 µL of Assay Buffer 18.
2. For each well, prepare 50 µL MAO-B Enzyme Solution:

	Enzyme Solution
Assay Buffer 18	49 µL
Diluted MAO-B Enzyme	1 µL

3. Mix. Add 50 µL/well into wells containing test inhibitors, Inhibitor Control, & Enzyme Control. Incubate for 10 minutes at 37°C.

Δ Note: *Always freshly prepare MAO-B Enzyme working solution. Don't store the enzyme working solution.*

Δ Note: *To check the possible inhibitory effect of test inhibitors on Developer Solution V, replace the 1 µL of diluted MAO-B Enzyme with 1 µL of 10 mM H_2O_2. Mix & add 50 µL to the TI well. Incubate for 10 minutes at 37°C.*

MAO Substrate Preparation:

1. For each well, prepare 40 µL of MAO Substrate:

	Substrate Solution
Assay Buffer 18	37 µL
MAO Substrate	1 µL
Developer Solution V	1 µL
OxiRed™ Probe	1 µL

2. Mix well and add 40 µL of the MAO Substrate into each well. Mix well.

Measurement

Measure the fluorescence (Ex/Em = 535/587 nm) kinetically at 37°C for 10-40 min. Choose two points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).

Calculation

1. Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2 - RFU_1$) values by the time Δt ($T_2 - T_1$). Calculate % Relative Inhibition as follows:

$$\% \text{ Relative Inhibition} = \frac{(Slope\ of\ EC - Slope\ of\ S)}{Slope\ of\ EC} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)