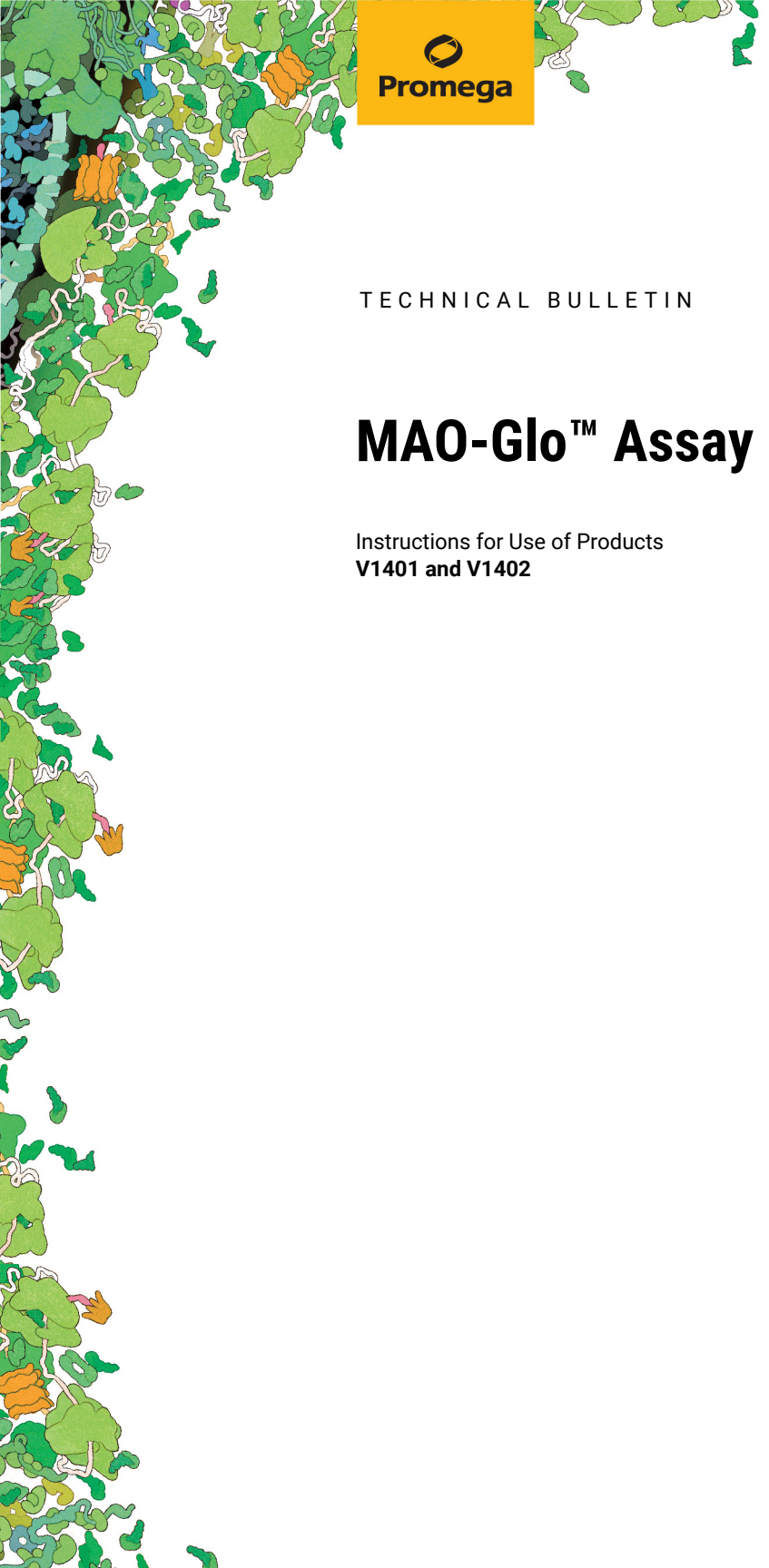

TECHNICAL BULLETIN

MAO-Glo™ Assay

Instructions for Use of Products
V1401 and V1402

Revised 12/23
TB345

MAO-Glo™ Assay

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description 1

2. Product Components and Storage Conditions 3

3. Performing the MAO-Glo™ Assay 3
- **3.A. General Considerations 3**
- **3.B. Reagent Preparation 5**
- **3.C. Protocol 6**

4. Results 6

5. Composition of Buffers and Solutions 7

6. References 7

7. Appendix 8
- **7.A. Monoamine Oxidase Reaction Chemistry 8**
- **7.B. Luciferin Detection Chemistry 12**
- **7.C. Measuring the Affinity of Known Substrates and Inhibitors 13**

8. Related Products 15

9. Summary of Changes 17

1. Description

The MAO-Glo™ Assay[a] provides a homogeneous luminescent method for measuring monoamine oxidase (MAO) activity from recombinant and native sources and measuring the effects of test compounds on MAO activities (1).
The MAO-Glo™ Assay is performed by incubating the MAO enzyme with a luminogenic MAO substrate. The substrate of the MAO-Glo™ Assay is a derivative of beetle luciferin ((4S)-4,5-dihydro-2-(6-hydroxybenzothiazolyl)-4-thiazolecarboxylic acid). MAO converts this luciferin derivative to methyl ester luciferin.

1. Description (continued)

After the MAO reaction has been performed, the reconstituted Luciferin Detection Reagent is added to simultaneously stop the MAO reaction, convert the methyl ester derivative to luciferin and produce light (Figure 1). This addition initiates a stable glow-type luminescent signal with a half-life greater than 5 hours. The amount of light produced is directly proportional to the activity of MAO.

Figure 1. Conversion of the MAO Substrate by MAO. Monoamine oxidase acts on the luminogenic MAO Substrate to produce methyl ester luciferin, which is then converted into light by the esterase and luciferase enzymes in the reconstituted Luciferin Detection Reagent. Any effects of test compounds on luciferase or esterase activities have been minimized (Section 7.B).

The MAO-Glo™ Assay includes a luminogenic MAO Substrate, two MAO Reaction Buffers (one that can be used with either MAO-A or MAO-B and one that is designed specifically for MAO-B), a lyophilized Luciferin Detection Reagent and the Reconstitution Buffer with esterase. The user supplies the sample material containing MAO. Protocols are configured for multiwell plate formats but can easily be adapted for single-tube applications.

The MAO-Glo™ Assay is ideal for many applications, including:

- Measuring native and recombinant MAO activities
- Screening drugs and chemical entities for their capacity to modulate MAO activities in native or recombinant fractions

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
MAO-Glo™ Assay	**200 assays**	**V1401**

The kit contains sufficient reagents for 200 assays at 50µl per assay in 96-well plates or 330 assays at 30µl per assay in 384-well plates. Includes:

- 1 vial MAO Substrate
- 10ml MAO Reaction Buffer
- 10ml MAO B Reaction Buffer
- 10ml Reconstitution Buffer with esterase
- 1 vial Luciferin Detection Reagent

PRODUCT	SIZE	CAT.#
MAO-Glo™ Assay	**1,000 assays**	**V1402**

The kit contains sufficient reagents for 1,000 assays at 50µl per assay in 96-well plates or 1,660 assays at 30µl per assay in 384-well plates. Includes:

- 1 vial MAO Substrate
- 50ml MAO Reaction Buffer
- 50ml MAO B Reaction Buffer
- 50ml Reconstitution Buffer with esterase
- 1 vial Luciferin Detection Reagent

Storage Conditions: Store all components at –30°C to –10°C protected from light. See expiration date on the product label. Avoid multiple freeze-thaw cycles of all components.

The resuspended MAO Substrate can be stored at –30°C to –10°C for up to 3 months. The reconstituted Luciferin Detection Reagent can be stored at room temperature (approximately 22°C) for 1 week or –30°C to –10°C for 2 months with no change in activity. The activity of the reconstituted Luciferin Detection Reagent decreased approximately 10% when the reagent was stored at +2°C to +10°C for 2 months.

3. Performing the MAO-Glo™ Assay

3.A. General Considerations

The MAO-Glo™ Assay is performed in two steps (Figure 2).

Step 1. The MAO Reaction: The first step of the MAO-Glo™ Assay combines the MAO Substrate with MAO enzyme to generate methyl ester luciferin. The recommended final substrate concentrations are the apparent K_m values for the respective reactions, and the recommended amount of MAO should give strong signals and high signal-to-background ratios. However, the magnitude of the MAO-Glo™ Assay signal will vary with specific activity of the MAO preparation. The MAO-Glo™ Assay is performed in MAO Reaction Buffer; MAO-B reactions may also be performed in MAO B Reaction Buffer, which contains 10% DMSO to increase the activity of MAO-B by approximately fourfold (Section 7.A).

3.A. General Considerations (continued)

Step 2. Luciferin Detection: The methyl ester luciferin, which is produced in Step 1 by the action of MAO on the MAO Substrate, reacts with esterase and luciferase to produce light in Step 2. The MAO-Glo™ Assay uses a proprietary stabilized luciferase (Ultra-Glo™ Luciferase) and a proprietary detection reagent formulation to produce a stable "glow-type" luminescent signal with a half-life greater than 5 hours (extrapolated from Figure 9, Section 7.B).

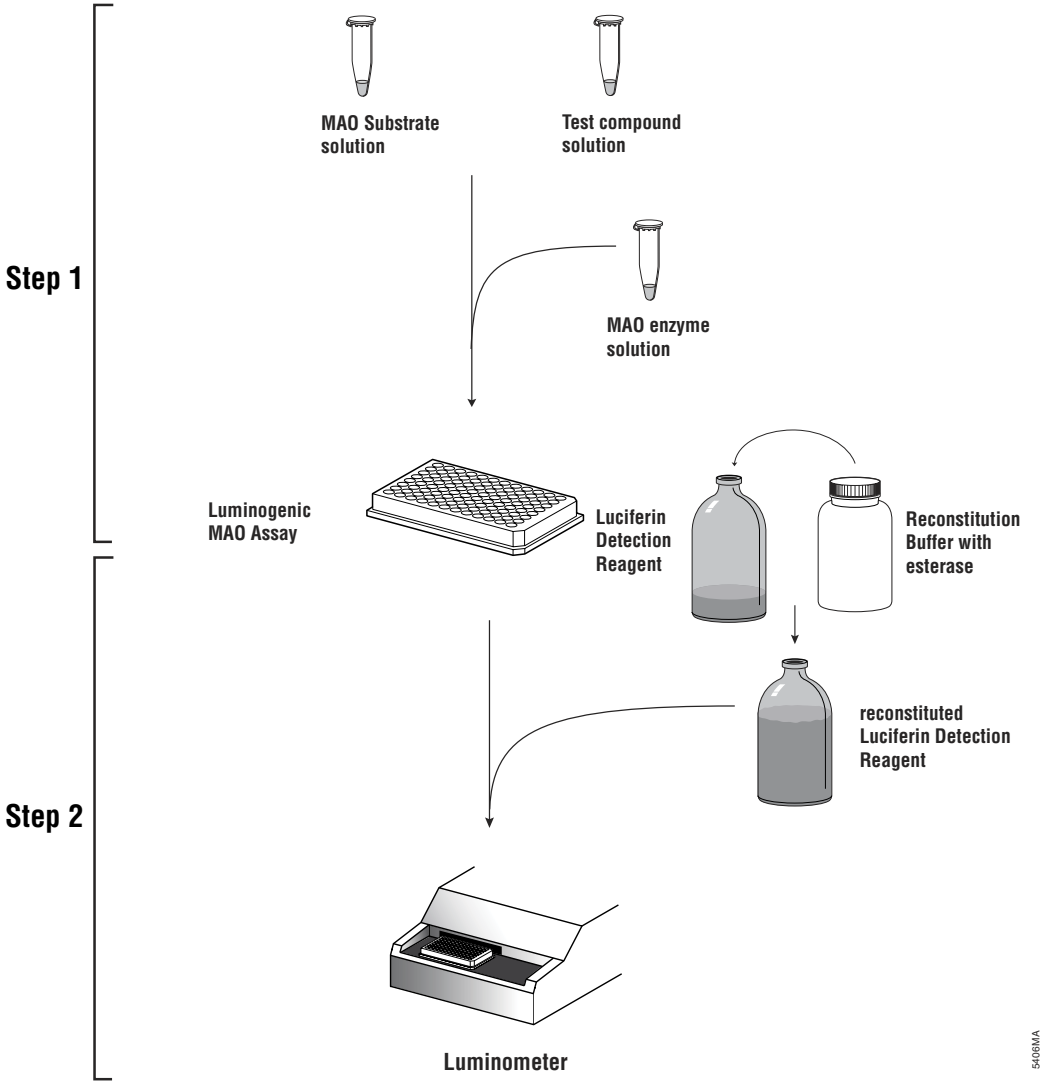

Figure 2. Schematic diagram showing reagent preparation and use of the MAO-Glo™ Assay.

3.B. Reagent Preparation

The resuspended MAO Substrate can be stored at –20°C for up to 3 months. The reconstituted Luciferin Detection Reagent can be stored at room temperature (approximately 22°C) for 1 week or –20°C for 2 months with no change in activity. The activity of the reconstituted Luciferin Detection Reagent decreased approximately 10% when the reagent was stored at 4°C for 2 months. Avoid multiple freeze-thaw cycles of all components.

Use the same reaction buffer, either MAO Reaction Buffer or MAO B Reaction Buffer, for each dilution within a single experiment.

Note: The volumes given here are intended for 96-well plates. For 384-well plates, we recommend using 60% of the volumes indicated.

Materials to Be Supplied by the User

- MAO enzyme preparation
- test compound
- dimethyl sulfoxide (DMSO)

Preparation of the 2X MAO Enzyme Solution

For each reaction, prepare 26.25µl of a 2X MAO enzyme preparation. This volume allows 5% extra material to compensate for pipetting error. Dilute the MAO-containing material with MAO Reaction Buffer to achieve the desired concentration.

Preparation of the 4X Test Compound

Prepare a 4X solution of the compound to be tested. Dilute or dissolve the test compound in MAO Reaction Buffer.

Preparation of the MAO Substrate Solution

1. Dissolve the dried MAO Substrate in DMSO. For Cat.# V1401, dissolve the substrate in 105µl of DMSO; for Cat.# V1402, dissolve the substrate in 525µl of DMSO. This will create a 4mM stock solution.
2. For each reaction, prepare 13.13µl of a 4X MAO Substrate solution. This volume allows 5% extra material to compensate for pipetting error. Dilute the resuspended MAO Substrate from Step 1 with MAO Reaction Buffer to a concentration of 160µM (25-fold dilution) to assay MAO-A or 16µM (250-fold dilution) to assay MAO-B.

   For example, to perform a 96-well plate of reactions for MAO-A, use 50µl of MAO Substrate diluted with MAO Reaction Buffer to a final volume of 1,250µl; if assaying MAO-B, use 5.0µl of MAO Substrate diluted to a final volume of 1,250µl.

Preparation of the Reconstituted Luciferin Detection Reagent

1. Equilibrate the Reconstitution Buffer with esterase and lyophilized Luciferin Detection Reagent to room temperature.
2. Transfer the entire contents of the bottle of Reconstitution Buffer with esterase to the amber bottle of lyophilized Luciferin Detection Reagent. Mix by swirling or inverting several times to obtain a homogeneous solution. Do not vortex.

3.C. Protocol

1. Add 12.5µl of 4X MAO Substrate solution per well.
2. Add 12.5µl of 4X test compound per well. If the assay is to be performed without a test compound, add 12.5µl of MAO Reaction Buffer containing the appropriate carrier or solvent.
3. To initiate the MAO reaction, add 25µl of 2X MAO enzyme solution per well, and mix briefly. For negative control reactions, add 25µl of MAO Reaction Buffer.
4. Incubate the plate at room temperature for 1 hour.

   Note: The net luminescent signal of the MAO-Glo™ Assay depends on both time and temperature (Section 7.A).
5. Add 50µl of reconstituted Luciferin Detection Reagent per well, and mix briefly.
6. Incubate the plate at room temperature for 20 minutes to generate and stabilize the luminescent signal.
7. Measure and record the luminescent signal using a plate-reading luminometer or CCD camera. Values are displayed as relative light units (RLU).

   Note: Luminometer settings will depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline. Relative light units are arbitrary units that vary, depending on the instrument manufacturer and model. Absolute readings from one luminometer may not match those from a different model.

4. Results

Calculate net MAO-dependent luminescence (net RLU) by subtracting the average luminescence of the negative control reactions without MAO enzyme from that of the MAO-containing reactions. The net signal from MAO reactions in the absence of test compound represents the total MAO activity. Changes from the average net signal for total MAO activity to the net signals for reactions with test compound reflect the effect of the compound on MAO activity. Changes in luminescent signal will typically be seen as decreases because of the inhibition of MAO or use of alternative substrates. However, some compounds may cause signals to increase if they activate the MAO enzyme.

The product of MAO and esterase enzyme activities is D-luciferin. D-luciferin reacts with luciferase to produce light that is directly proportional to the D-luciferin concentration (Figure 3). For example, an inhibitor concentration that causes a 50% drop in luminescence has also caused a 50% decrease in D-luciferin accumulation. Data may be expressed directly in relative light units (RLU) or percent activity, or the values may be converted to reaction rates by dividing RLU by reaction time and amount of enzyme. For example, obtaining 100,000 RLU from a 20-minute reaction with 0.5pmol of MAO-A corresponds to a specific activity of 10,000 RLU/pmol MAO-A/minute. Alternatively, RLU may be converted to a corresponding D-luciferin concentration for assays that include a D-luciferin standard curve. However, to detect MAO inhibition at a single concentration of a test compound or to measure the IC_{50}, K_i or K_m value for an inhibitor or substrate, it is not necessary to convert luminescence to D-luciferin concentration.

Note: IC_{50} refers to the concentration of a compound that inhibits a reaction by 50%. In the case of competitive inhibition, $IC_{50} = 2K_i$ when the substrate is present at the K_m concentration, as per the relationship:
$K_i = IC_{50}/[1 + (\text{substrate concentration}/K_m)]$.

If IC_{50} values obtained with the MAO-Glo™ Assay will be compared to IC_{50} or K_i values obtained with other MAO assays, you must consider this relationship and note that direct comparisons should only be made when the respective substrates are present at their K_m concentrations.

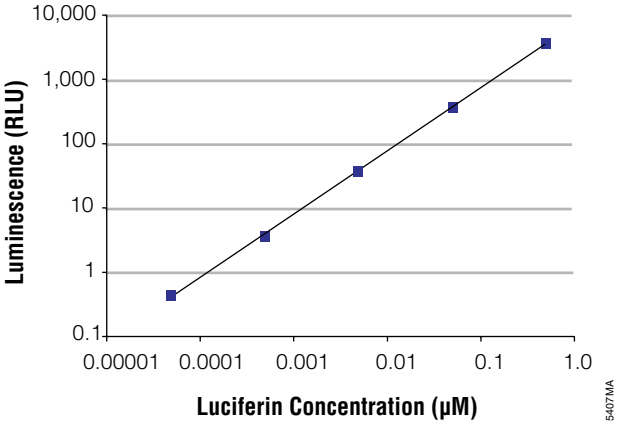

Figure 3. The correlation between luminescent signal and D-luciferin concentration. Luciferin was titrated in MAO Reaction Buffer and mixed with an equal volume of reconstituted Luciferin Detection Reagent. The luminescent signal was measured 20 minutes after reagent addition. For all samples n = 4, and the standard deviation is smaller than the data points shown.

5. Composition of Buffers and Solutions

MAO Reaction Buffer

100mM	HEPES (pH 7.5)
5%	glycerol

MAO B Reaction Buffer

100mM	HEPES (pH 7.5)
5%	glycerol
10%	dimethyl sulfoxide

6. References

1. Valley, M. *et al.* (2006) A bioluminescent assay for monoamine oxidase activity. *Anal Biochem.* **359**, 238–46.
2. Johnston, J.P. (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **17**, 1285–97.
3. Tsugeno, Y. *et al.* (1995) Regions of the molecule responsible for substrate specificity of monoamine oxidase A and B: A chimeric enzyme analysis. *J. Biochem.* **118**, 974–80.
4. Schoepp, D.D. and Azzaro, A.J. (1981) Specificity of endogenous substrates for types A and B monoamine oxidase in rat striatum. *J. Neurochem.* **36**, 2025–31.
5. Tipton, K.F., Fowler, C.J. and Houslay, M.D. (1982) In: *Monoamine oxidase: Basic and clinical frontiers*, Kamijo, K., Usdin, E. and Nagatsu, T., eds., *Excerpta Medica*, Amsterdam, 87–99.
6. Geha, R.M. *et al.* (2001) Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *J. Biol. Chem.* **276**, 9877–82.

7. Appendix

7.A. Monoamine Oxidase Reaction Chemistry

Monoamine oxidase (MAO) catalyzes the oxidative deamination of amine-containing substrates. In the MAO-Glo™ Assay, an aminopropylether analog of methyl ester luciferin is provided as a substrate for the MAO reaction. In Step 1 (Figure 4, reactions 1–3), the MAO enzyme oxidizes the amine to an imine, which is subsequently hydrolyzed by water to the corresponding aldehyde. The aldehyde then spontaneously undergoes a β-elimination reaction to generate methyl ester luciferin. Since the latter two reactions are not rate-limiting, the amount of methyl ester luciferin produced is proportional to the activity of MAO.

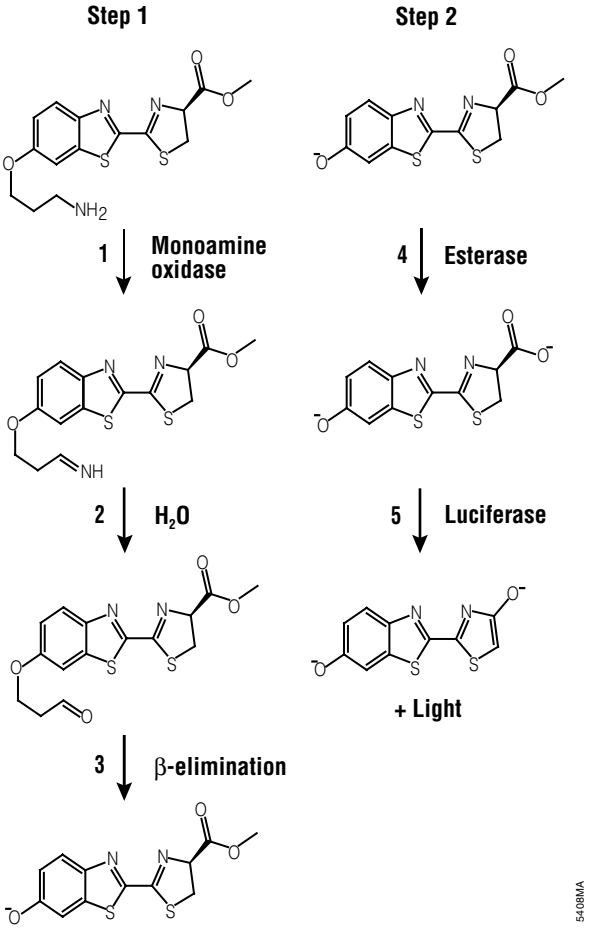

Figure 4. The chemical and enzymatic reactions required to generate light in the MAO-Glo™ Assay.

The MAO-A and MAO-B enzymes both use the MAO Substrate, but the two enzymes have different K_m and RLU_{max} values (Figure 5). We recommend performing the MAO reactions with the MAO Substrate at their individual K_m values of 40µM and 4µM (MAO-A and MAO-B, respectively).

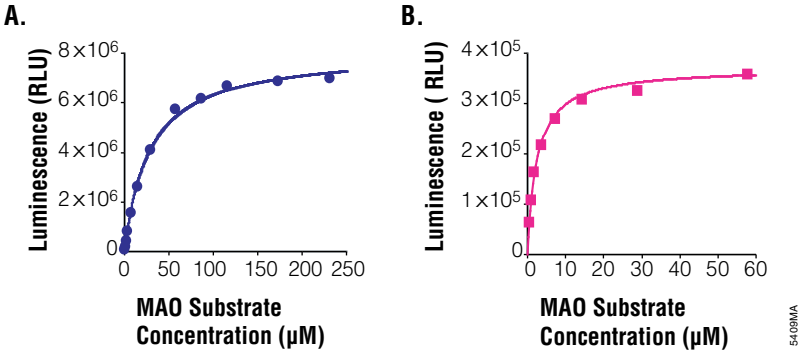

Figure 5. K_m values for the MAO Substrate. MAO-A reactions were performed in MAO Reaction Buffer (**Panel A**), and MAO-B reactions were performed in MAO-B Reaction Buffer (**Panel B**) for 1 hour at 25°C. Microsomes containing the MAO-A or MAO-B enzymes (Sigma-Aldrich, Inc., Cat.# M7316 and M7441, respectively) were used at 1µg of protein per 50µl reaction. Addition of an equal volume of reconstituted Luciferin Detection Reagent terminated the MAO reaction and generated a luminescent signal, which was read 20 minutes after addition. The data were fit (solid lines) with the program TableCurve (Systat Software, Inc.).

The amount of MAO used and the reaction time will affect the level of luminescence (Figure 6). Increasing the length of the MAO reaction permits the use of less MAO enzyme per reaction. Conversely, increasing the amount of MAO enzyme may permit reaction times as short as 10–20 minutes. We recommend running MAO reactions with 1µg of protein from microsomes containing MAO for 1 hour, but the amount of enzyme and time should be optimized for each application. For example, reactions with 0.2µg, 1µg or 5µg of protein incubated for 3 hours, 40 minutes or 10 minutes, respectively, all give signal-to-noise ratios greater than 100-fold.

7.A. Monoamine Oxidase Reaction Chemistry (continued)

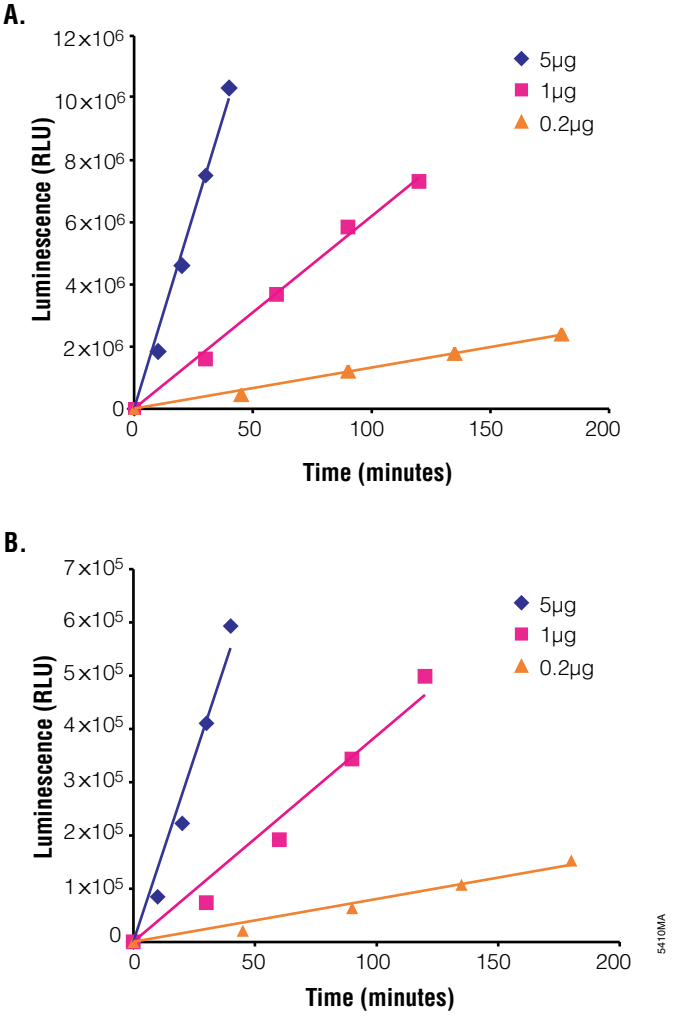

Figure 6. The luminescent signal varies with the amount of enzyme and time in Step 1. The MAO Substrate (40µM for MAO-A or 4µM for MAO-B) was incubated for various lengths of time with microsomes containing MAO-A (**Panel A**) or MAO-B (**Panel B**). Each 50µl reaction contained 5, 1 or 0.2µg of protein. The luminescent signal was measured 20 minutes after adding the reconstituted Luciferin Detection Reagent. For all samples, n = 3, and the standard deviation is smaller than the data points shown.

MAO-A and MAO-B are affected differently by environmental conditions. Although solvents such as DMSO are detrimental to the MAO-A reaction, low concentrations of solvent increase the reaction rate of MAO-B (Figure 7) without significantly affecting its K_m value for the MAO Substrate (data not shown). For this reason, the MAO Reaction Buffer is a general formulation that can be used with either MAO enzyme, whereas the MAO-B Reaction Buffer, which contains 10% DMSO, has been optimized to maximize the luminescent signal in MAO-B reactions. Temperature also has a much greater effect on the MAO-B reaction compared to the MAO-A reaction, such that increasing the temperature of the MAO-B reaction can yield a significant increase in the net luminescent signal (Figure 8).

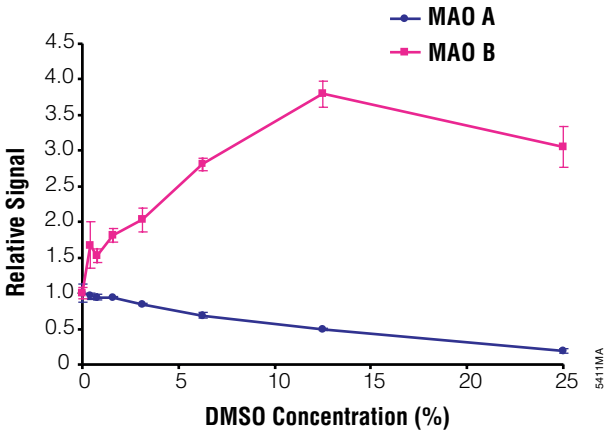

Figure 7. The effect of DMSO concentration on MAO activity. The MAO Substrate (40µM or 4µM) was incubated for 1 hour in MAO Reaction Buffer with microsomes containing MAO-A or MAO-B (1µg of protein per 50µl reaction), respectively, and various concentrations of DMSO. The luminescent signal was measured 20 minutes after adding the reconstituted Luciferin Detection Reagent. The relative signal values were normalized to the luminescent signal in the absence of DMSO. For all samples, n = 3, and the standard deviation is illustrated by the error bars shown.

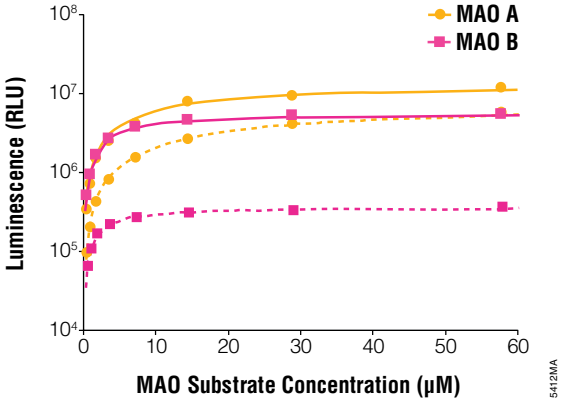

Figure 8. The effect of temperature on MAO activity. Reaction conditions are the same as those in Figure 5, except the MAO-A and MAO-B reactions were performed at 25°C (dotted lines) and 35°C (solid lines).

7.B. Luciferin Detection Chemistry

In Step 2, the reconstituted Luciferin Detection Reagent inactivates MAO and provides both the enzymes and cosubstrates necessary to convert the product of Step 1 into a luminescent signal. As shown in Figure 4 (reactions 4–5), an esterase converts the methyl ester luciferin into luciferin, which is subsequently consumed by luciferase to yield oxyluciferin and light. Since time is required for the esterase reaction and stabilization of the luciferase signal, the reconstituted Luciferin Detection Reagent should be added to the plates at least 20 minutes before quantifying luminescence (Figure 9). After this period, the luminescent signal is extremely stable, allowing the quantitation of numerous plates of samples at any time over many hours. For the most consistent data, we recommend incorporating a common control on each plate to account for any small variations in luminescence that can occur over time, such as those due to changes in temperature or the instrument signal.

The potential for the luciferase and esterase in the MAO-Glo™ Assay to affect the outcome of an assay has been minimized by maintaining high enzyme concentrations and creating specific chemistries to reduce the effects of potential inhibitors. For example, 10µM of the esterase substrates ethyl butyrate, ethyl acetate and 4-nitrophenyl acetate had little to no effect on the luminescent output of a MAO-B reaction (98.5% + 2.1%; 98.8% + 1.1%; and 98.4% + 1.6% of control samples, respectively; n = 3).

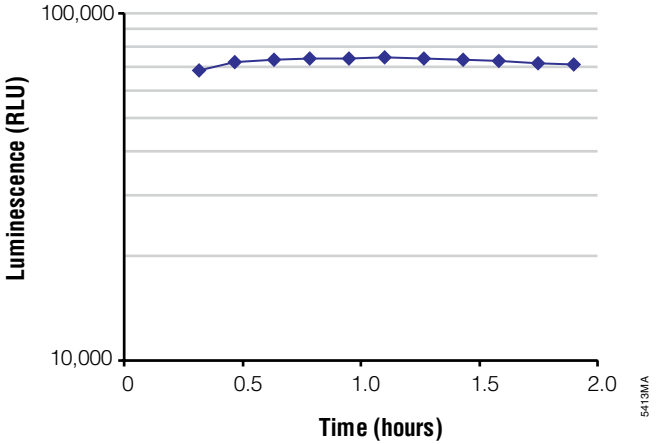

Figure 9. The luminescent signal is stable in Step 2. In a 50µl reaction, 40µM MAO Substrate was incubated with 2µg of protein from microsomes containing MAO-A for 45 minutes. After adding an equal volume of reconstituted Luciferin Detection Reagent, the luminescent signal was read repeatedly over 2 hours. Similar trends were noted in experiments with MAO-B.

7.C. Measuring the Affinity of Known Substrates and Inhibitors

The ability of the MAO-Glo™ Assay to detect the effect of test compounds on MAO activity was demonstrated by performing the assay in the presence of known substrates or inhibitors of MAO enzymes (Figure 10). Clorgyline and deprenyl are MAO-A- and MAO-B-specific inhibitors, respectively, while phenylethylamine, serotonin and dopamine are substrates with varying specificity for each MAO isozyme. In Table 1, the K_m or K_i values obtained with the MAO-Glo™ Assay are compared to those previously published. The values for known substrates or inhibitors shown in Table 1 were calculated from the data shown in Figure 10.

Table 1. K_i or K_m Values for Known MAO Substrates or Inhibitors.

	MAO-A		MAO-B	
	K_i or K_m value (µM)	**published value (µM)**	**K_i or K_m value (µM)**	**published value (µM)**
clorgyline	0.003 ± 0.001	0.0039[1]	10 ± 4	4[1]
deprenyl	7 ± 1	5[2]	0.5 ± 0.2	0.13[2]
phenylethylamine	78 ± 16	78[3]	16 ± 1	20[4]
serotonin	45 ± 8	80[5]	410 ± 140	2032[4]
dopamine	21 ± 1	120[3]	570 ± 120	301[3]

[1]As published by Johnston (2).

[2]As published by Tsugeno *et al.* (3).

[3]As published by Schoepp and Azzaro (4).

[4]As published by Tipton, Fowler and Houslay (5).

[5]As published by Geha *et al.* (6).

7.C. Measuring the Affinity of Known Substrates and Inhibitors (continued)

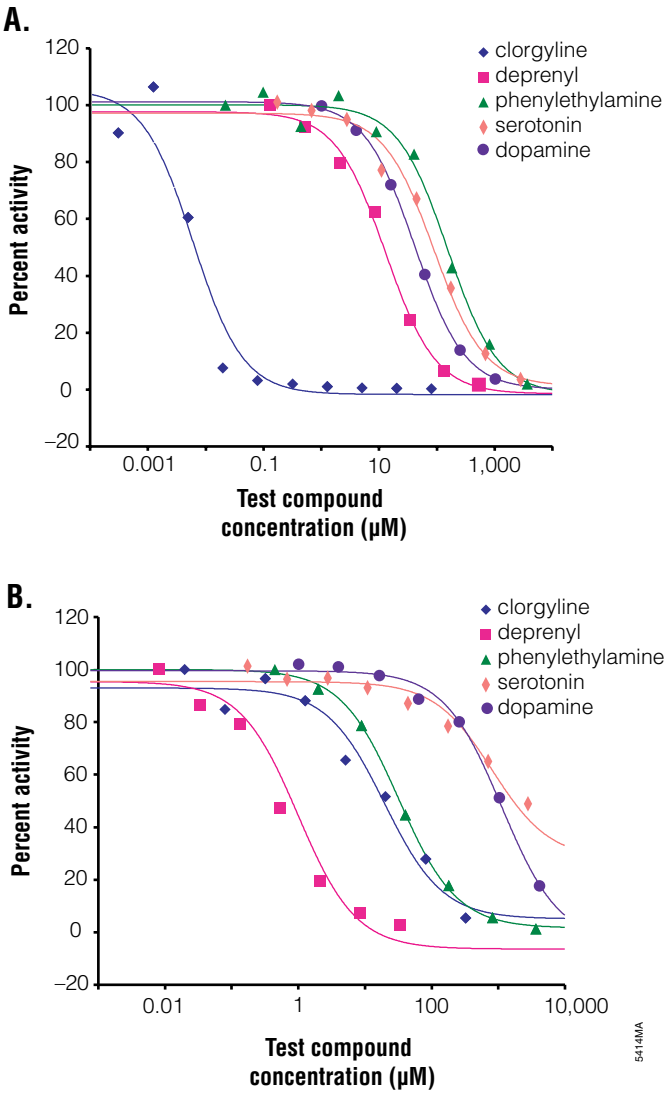

Figure 10. The MAO-Glo™ Assay can measure the binding of known substrates and inhibitors. MAO-A (**Panel A**) and MAO-B (**Panel B**) reactions were performed in the presence of various inhibitors for 1 hour at 25°C, and the luminescent signal was measured 20 minutes after adding the reconstituted Luciferin Detection Reagent. The data was fit (solid lines) with the program TableCurve (Systat Software, Inc.). Test compounds were purchased from Sigma-Aldrich, Inc., and dissolved in MAO Reaction Buffer prior to use.

8. Related Products

Cytochrome P450 Assays

Product	Size	Cat.#
P450-Glo™ CYP1A1 Assay	50ml	V8752
P450-Glo™ CYP1B1 Assay	50ml	V8762
P450-Glo™ CYP1A2 Assay	50ml	V8772
P450-Glo™ CYP2C8 Assay	50ml	V8782
P450-Glo™ CYP2C9 Assay	50ml	V8792
P450-Glo™ CYP3A4 Assay	50ml	V8802
P450-Glo™ CYP3A7 Assay	50ml	V8812
P450-Glo™ CYP2C19 Assay	50ml	V8882
P450-Glo™ CYP2D6 Assay	50ml	V8892

Additional 10ml size available.

Cytochrome P450 Screening Systems

Product	Size	Cat.#
P450-Glo™ CYP1A2 Screening System	1,000 assays	V9770
P450-Glo™ CYP2C9 Screening System	1,000 assays	V9790
P450-Glo™ CYP3A4 Screening System	1,000 assays	V9800
P450-Glo™ CYP2C19 Screening System	1,000 assays	V9880
P450-Glo™ CYP2D6 Screening System	1,000 assays	V9890

Proteasome Assays

Product	Size	Cat.#
Proteasome-Glo™ Chymotrypsin-Like Assay	10ml	G8621
Proteasome-Glo™ Trypsin-Like Assay	10ml	G8631
Proteasome-Glo™ Caspase-Like Assay	10ml	G8641
Proteasome-Glo™ 3-Substrate System	10ml	G8531

Additional sizes available.

Promega

8. Related Products (continued)

Oxidative Stress Assays

Product	Size	Cat.#
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
Mitochondrial ToxGlo™ Assay	10ml	G8000

Additional sizes available.

Cell Viability, Cytotoxicity and Apoptosis Assays

Product	Size	Cat.#
CellTiter-Glo® 2.0 Cell Viability Assay	10ml	G9241
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
RealTime-Glo™ MT Cell Viability Assay	100 assays	G9711
CellTox™ Green Cytotoxicity Assay	10ml	G8741
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
Caspase-Glo® 3/7 Assay System	2.5ml	G8090
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011

Additional sizes available.

Luminometers

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax® Explorer System	1 each	GM3500

9. Summary of Changes

The following changes were made to the 12/23 revision of this document:

1. Updated Section 8 and patent statements.
2. Changed font and cover image.
3. Made minor text edits.

[a]Patent Pending.