MAO-Glo™ Assay: Fast and Easy Luminescent Assay to Measure Monoamine Oxidases

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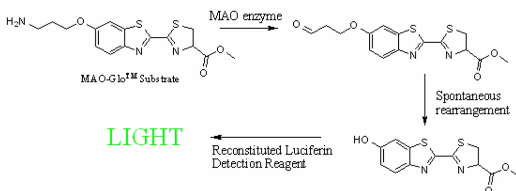
Abstract

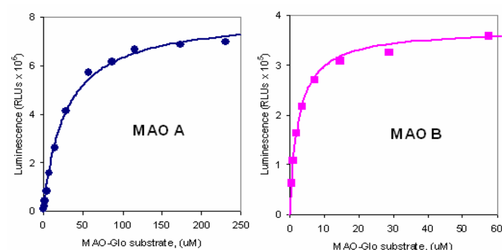
The MAO-Glo™ Assay, a luminescent method for sensitive quantitation of monoamine oxidase (MAO) activity, enables rapid assessment of potential inhibition caused by new chemical entities. The homogeneous assay format is compatible with laboratory automation, or can be performed manually (*e.g.*, 96 samples in under 90 minutes). The assay is suitable for both MAO A and MAO B, and data generated for known MAO inhibitors yielded K_i and IC_{50} values consistent with published literature. The signal-to-noise ratios is >1000 and Z' values ~ 0.95.

Assay Concept

Monoamine oxidases (MAO) play an important role in the cytotoxicity and metabolism of xenobiotic and biogenic amine-containing compounds, and thus can drastically affect the therapeutic drug content within cells. To enable rapid quantitation of MAO activity, luciferin analogs were synthesized with amine-containing moieties that upon modification by MAO result in light production in the presence of luciferase. The MAO-Glo Substrate (shown below) exhibits a high level of specificity with both MAO A and MAO B.

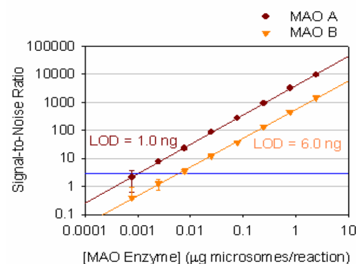


Assay Method



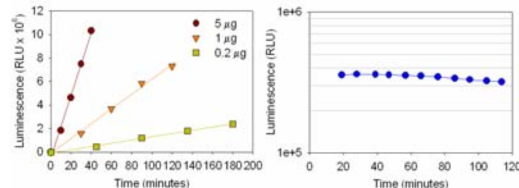
Assays are performed by adding MAO-Glo™ substrate to microsomes containing MAO enzymes (typically ~1 µg microsomes per 50 µl reaction at 25 ºC in 100 mM HEPES, pH 7.5 with 5% glycerol; MAO-B reaction contained also 10% DMSO). After 1 hour, an equal volume of Reconstituted Luciferin Detection Reagent is added and the luminescent signal measured after 20 minutes. All subsequent experiments were performed at K_m.

Assay Sensitivity and Linearity



Light intensity of the MAO-Glo™ Assay is proportional to enzyme activity over >10,000-fold range. The signal:noise ratio was determined by dividing the net luminescent signal by the standard deviation of the background. The level of detection (LOD) is the concentration of enzyme required to generate a signal:noise ratio of 3 (blue line).

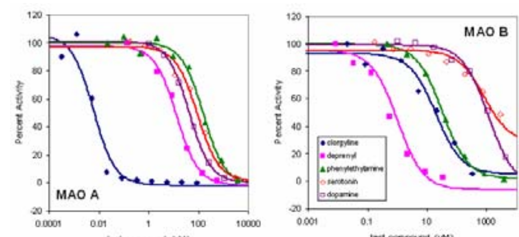
Assay Kinetics



Left graph: The luminescent signal is proportional to MAO enzyme concentration and time. The MAO-Glo substrate was incubated with MAO A microsomes at 5, 1 or 0.2 µg per 50 µl reaction, and the luminescent signal was measured 20 minutes after addition of the Reconstituted Luciferin Detection Reagent.

Right graph: The luminescent signal is nearly constant over time. The MAO A reaction (2 µg of microsomes per 50µl reaction for 45 minutes) was measured repeatedly after adding the Reconstituted Luciferin Detection Reagent. Similar trends were noted in similar experiments with MAO B.

Response to Known MAO Inhibitors



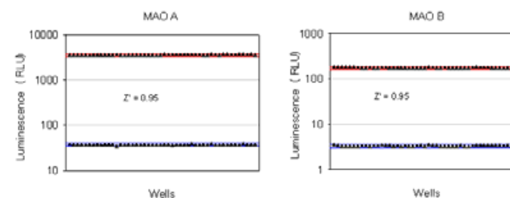
Values for K_i or K_m of known inhibitors and substrates can be determined by inhibition of the luminescent reaction. Clorgyline and deprenyl are MAO A and MAO B specific inhibitors, respectively, while phenylethylamine, serotonin, and dopamine are substrates with varying specificity for each MAO enzyme.

Agreement with Published K_i and K_m Values

	MAO A		MAO B	
	K_i or K_m (µM)	published value (µM)	K_i or K_m (µM)	published value (µM)
clorgyline	0.003 ± 0.001	0.025[d]	10 ± 4	79[d]
deprenyl	7 ± 1	5[d]	0.5 ± 0.2	0.13[d]
phenylethylamine	78 ± 16	78[c]	16 ± 1	20[a]
serotonin	45 ± 8	80[b]	410 ± 140	2032[a]
dopamine	21 ± 1	120[c]	570 ± 120	301[c]

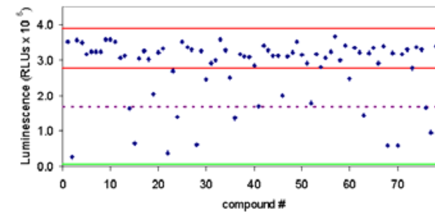
[a]Tipton, K.F., Fowler, C.J., and Houslay, M.D. (1982) *Monoamine Oxidase: Basic and Clinical Frontiers* (Kamijo, K., Usdin, E., and Nagatsu, T., Eds.), p. 87-99, Excerpta Medica, Amsterdam. [b]Geha, R.M., Rebrin, I., Chen, K., and Shih, J.C. (2001) *Journal of Biological Chemistry*, 276(13): 9877-9882. [c]Schoepp, D.D., and Azzaro, A.J. (1981) *Journal of Neurochemistry*, 36(6): 2025-2031. [d]Tsugeno, Y., Hirashiki, I., Ogata, F., and Ito, A. (1995) *Journal of Biochemistry*, 118: 974-980.

Assay Precision (Z' values)



Assays of MAO A and MAO B in 96-well plates both gave Z' values of ~0.95. (Data collected from plates where half the wells contained 2 µg microsomes/reaction, and the remaining wells were filled with MAO Reaction Buffer) Assays in 384-well plates gave Z' values of up to 0.95 for MAO A and 0.89 for MAO B.

LOPAC Screen



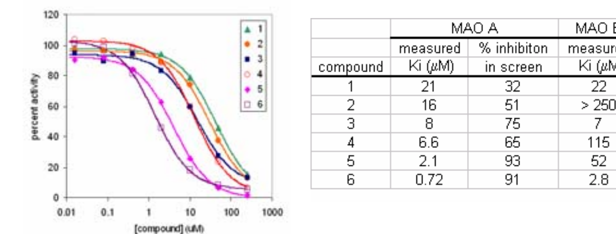
Screening the expanded LOPAC library (Sigma Chemical, Saint Louis) indicated 153 compounds that interacted with MAO A. Each of the compounds (10 µM) was added to the MAO enzymes (0.5 µg microsomes per reaction) and the MAO-Glo™ substrate. Shown above is LOPAC plate 13; the solid red and dashed lines indicate 15% and 50% deviations from the average of control wells with no compound addition, respectively, and the green line indicates the average of control wells with no MAO enzyme.

Consistency with Literature

compound	action	% inhibition
hydralazine	inhibitor	29
tryptamine	substrate	60
O-methylserotonin	substrate	71
amiloride	inhibitor	82
phenelzine	inhibitor	92
Ro 41-1049	inhibitor	95
clorgyline	inhibitor	98

Data concerning compounds from the LOPAC library known as MAO A inhibitors or substrates were extracted from the screen.

Confirmation of Screening Hits



	MAO A		MAO B
	measured	% inhibiton	measured
compound	Ki (µM)	in screen	Ki (µM)
1	21	32	22
2	16	51	> 250
3	8	75	7
4	6.6	65	115
5	2.1	93	52
6	0.72	91	2.8

To confirm the apparent inhibition of MAO activity, titration analysis was performed on selected compounds using MAO A and MAO B. Six compounds were chosen from the 153. These six were interesting because they did not contain nitrogen and thus should not compete as substrates for MAO. These 6 were also found not to affect the Reconstituted Luciferin Detection Reagent.

Summary

- MAO-Glo™ Assay is a simple, homogeneous assay reagent system for measuring MAO A or MAO B.
- Signal:noise ratios exceed 1000 and Z'-value is ~0.95.
- Known inhibitors and substrates perform as predicted by the literature.
- Several MAO A enzyme effectors were found in the LOPAC library, some of which were known in the literature.