

Fluoro: MAO™

Monoamine Oxidase A & B Detection Kit

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Notes

Revised protocol 5/06
Updated 1/07
Updated 9/2011: Storage Conditions

I. Assay Principle:

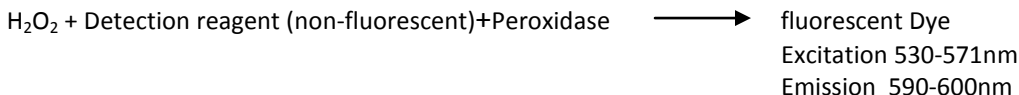
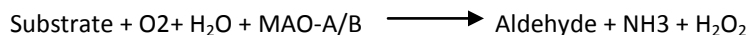
Monoamine oxidase (MAO) is a flavin-containing enzyme that catalyses the oxidation of a variety of amine-containing neurotransmitters such as serotonin, norepinephrine, epinephrine and dopamine to yield the corresponding aldehydes (1). MAO exists in two isoforms, namely MAO-A and MAO-B, which are the products of two distinct genes (2).

MAO-A and B exhibit different specificities to substrates and inhibitor selectivities. Extensive studies have been performed to characterize their properties (3-7). MAO-A acts preferentially on serotonin and norepinephrine, and is inhibited by clorgyline. MAO-B acts preferentially on 2-phenylethylamine and benzylamine and is inhibited by deprenyl and pargyline.

Localized in the outer mitochondrial membrane, these enzymes are found throughout the body. Often only one form of the enzyme is present in a specific organ and/or within a specific cell type (8-9). In addition to their role in regulating neurotransmitters, these enzymes are also involved in processing biogenic amines (10) including tyramine (11).

The Fluoro MAO-A/B detection kit utilizes a non- fluorescent substrate (Detection Reagent), to detect H₂O₂ released from the conversion of a substrate to its aldehyde via MAO-A/B. Furthermore H₂O₂ oxidizes the detection Reagent in a 1:1 stoichiometry to produce the fluorescent product. This oxidation is catalyzed by Peroxidase.

Reaction:



II. Storage:

1. The kit contains multiple storage temperature components. Please see labels of individual components for storage instructions.
2. Once a vial of the Detection reagent is opened, it should be used promptly and frozen since it is subject to oxidation by air.

III. Warnings and Precautions:

1. For Research use only. Not for use in diagnostic procedures.
2. Practice safe laboratory procedures by wearing protective clothing and eyewear.
3. The fluorescent product of the detection reagent is not stable in the presence of thiols (DTT or 2-mercaptoethanol). Keep these reactants below 10°C. ☐M. If you are using
reaction between pH 7-8 (optimal pH 7.4).
4. NADH and glutathione (reduced form: GSH) may interfere with the assay.
See Technical note #3.

IV. Part # 5018. Kit contents and Storage (for 500 assays).

Unopened kit may be stored at 4-8°C for 1 week. Refer to individual components for long-term storage conditions.

Description	Part#	Storage after opening Kit or Reconstitution of Reagents.
1 Bottle: 5X Reaction Buffer pH 7.4	3020	4-8 ^o C
1 vial: Detection reagent	4009	Aliquot in single use vials: Below -20 ^o C
1 vial: Horseradish Peroxidase	6007	4-8 ^o C
1 vial: MAO-B substrate Benzylamine	7006	Aliquot in vials: Below 4-8 ^o C
1 vial: MAO-A/B substrate: Tyramine	7005	Aliquot in single use vials: Below 4-8 ^o C
1 vial Pargyline: Monoamine Oxidase B inhibitor ⁽¹²⁻¹⁴⁾	7003	Aliquot in vials: Below -20 ^o C
1 vial Clorgyline: Monoamine Oxidase A inhibitor ⁽¹²⁻¹⁴⁾	7002	Aliquot in single use vials: Below 4-8 ^o C

Materials required but not supplied:

1. Dimethyl sulfoxide (DMSO)
2. Black 96-well plates
3. Fluorescence plate reader
4. Deionized water
5. Resorufin (optional - Sigma Cat# 424455 or R3257. Consult Sigma on how to dissolve the dye).

V. Preparation of reagent working solutions:

1. **1 X Reaction Buffer part# 3020:** To prepare a 1X solution of Reaction buffer, dilute the 5X buffer 1:5 with Di water. For example to prepare 20 ml of 1X Reaction buffer add 4ml of 5X Reaction buffer to 16ml of deionized water.
2. **Horseradish Peroxidase part# 6007.** Make a 100X stock solution of HRP by diluting it 1:18.9 with 1X Reaction Buffer. For example take out 5.29 L of the HRP reagent Reaction Buffer. Make enough HRP for 1 days worth of experiments.
3. **Detection Reagent Part# 4009:** Dissolve the contents of the vial with 500 μ L of DMSO. Allow the contents to sit at Room Temperature for 15 minutes. Next gently pipette up and down several times to dissolve any clumps. Once dissolved the detection reagent should be used promptly and any remaining reagent can be aliquoted and frozen at or below -20^oC. Avoid repeated freeze thaw cycles.
Note: Protect the Detection Reagent from light.
4. **Substrate: Benzylamine Part# 7006 and Tyramine Part# 7005.** Make a .5M solution by adding 0.6 mL of Di water to each vial. Allow the vial to sit at room temperature for 15 minutes. The vial should be vortexed to completely dissolve the solid.
5. **Inhibitor: Pargyline Part# 7003 and Clorgyline Part# 7002.** Make a 1mM solution by reconstituting the Vial with 0.2mL of Di water. Gently vortex the vial and allow the contents to dissolve at room temperature for 15-20 minutes.

VI. Assay Protocol: Detection of Monoamine oxidase A/B in Samples.

The following protocol is a general guide for adapting the Fluoro: MAO kit to detection monoamine oxidase activity in your samples. Substrate, inhibitor concentrations and time points should be optimized for your particular experimental conditions. The two monoamine oxidase substrates are interchangeable in the protocol. Tyramine is a substrate for monoamine oxidase A and B, while Benzylamine is a substrate for monoamine oxidase B.

Specific monoamine oxidase activity can be determined by combining substrates and inhibitors.

1. Prepare 10ml reaction cocktail for 100 assays:

100 μ L Detection Reagent .

100 μ L HRP of 100X HRP.

100 μ L of Substrate.

9.7ml of 1X Reaction buffer

The volume of the **reaction cocktail** can be scaled down or up (as needed) provided that the **ratios** of the ingredients are kept constant.

Note: See *Technical note #1*.

2. Preparation of samples.

Samples should be diluted in 1X Reaction Buffer. To measure specific monoamine oxidase activity their respective inhibitors can be added to each well at a final concentration between 0.5-10 μ M¹³. To measure total monoamine oxidase activity, omit adding any inhibitors. 100 μ L of sample volume will be used per test.

Note: Each investigator should optimize inhibitor concentration for their particular enzyme reaction. See Technical note 2.

3. Assay.

1. To a black 96 well plate pipette 100 μ L of samples to individual wells.

2. Add 100 μ L of Reaction Cocktail prepared in Section VI st

3. Incubate the sample at Room Temperature for 30-60 minutes.

Note: Each investigator should optimize the incubation time for their particular application.

4. Optional: A standard curve can be prepared from resorufin to determine moles of product produced. The standard curve can be constructed, using the 1X Reaction Buffer, from 0 – 50 μ M. Pipette 200 μ L of this standard curve to individual wells, prior to reading your samples.

Note: Each investigator should optimize the standard curve for their particular application.

5. Read samples using excitation: 530-570nm (570nm is optimal) and measure fluorescence at 590-600nm.

Note: See *Technical note 2*.

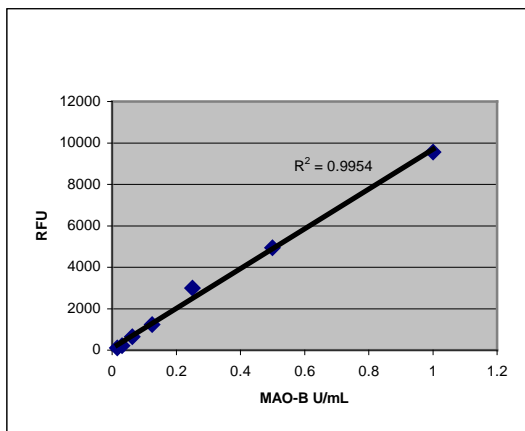


Figure 1. Recombinant MAO-B was serially diluted in 1X Reaction Buffer. 100 uL of diluted MAO-B per well was mixed with 100uL of Reaction Cocktail. The reaction was incubated at Room Temperature in the dark for 1 hour. Fluorescence was measured with a microplate reader using excitation at 530mM and fluorescence emission at 590mM.

VIII. Technical Notes:

- Final concentration of the substrate in the well will equal 2.5mM. Each investigator should titrate the substrates to optimize the kinetic reaction for their particular reaction.
Reported values for Substrates

Substrate	Monoamine Oxidase A: Substrate Concentration	Monoamine Oxidase B: Substrate Concentration
Tyramine ¹³	0.5mM (final)	2.5mM (final)
Benzylamine	NA	2.5mM

- The fluorescent product of the detection reagent is no stable in the presence of thiols (DTT or 2-mercaptoethanol). Keep these reactants below 10 μ M.
If you are using your own buffer, keep the reaction between pH 7-8 (optimal pH 7.4).
- At NADH levels above 10 μ M, detection reagent is unstable. This could result in aberrant readings. To minimize this interference it is recommended to add superoxide dismutase (SOD) at 40U/mL to the reaction¹⁵.
At glutathione (reduced form GSH) above 300 μ M, detection reagent is unstable. To minimize this interference it is recommended to add superoxide dismutase (SOD) at 40U/mL to the reaction¹⁵.

References:

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