Monoamine Oxidase A ELISA Kit – Format II
96T

General reference manual. Use the protocol shipped with the kit for your experiments.

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Reference manual for parts
- hMAO-A ELISA
- mMAO-A ELISA
- rMAO-A ELISA
- pMAO-A ELISA

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Store: 4°C. Expiration: 6 months after arrival.
Safety: Stop solution contains acid. Avoid all contact and inhalation. Wear eye protection.

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Monoamine Oxidase A ELISA Kit

Part No. hMAO-A-ELISA

For research only. Not for diagnostic applications.

Storage: 4°C, six months after arrival

Safety: Stop solution contains acid. Avoid eye and skin contact

Standard Peptides:
- Human: 450 ng/ml
- Mouse: 270 ng/ml
- Rat: 270 ng/ml
- Porcine: 128 ng/ml

Assay Ranges
- Human: 10 ng/ml - 400 ng/ml
- Mouse: 4ng/ml – 220 ng/ml
- Rat: 4ng/ml – 220 ng/ml
- Porcine: 0.5ng/ml – 100ng/ml

Please read the protocol prior to performing the assay

Experimental Principles

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to measure human Monoamine Oxidase (MAO-A) concentration. Samples are applied in microelisa wells pre-coated with an affinity purified polyclonal anti- Human MAO-A antibody. Samples are incubated and then washed. A second goat anti- MAO-A -HRP conjugate antibody is added, followed by incubation and wash. Chromogen solutions A and B are added resulting in a coloration change to blue. A stop solution is applied to terminate the reaction, turning the solution to yellow. Absorbance readings at 450 nm corresponding to standard peptide concentrations is used to correlate the concentration of MAO-A in the samples.

The ELISA kit detects MAO-A in a variety of sources. The following is provided as a generic guide to prepare the samples. The researcher should undertake a comprehensive overview of the literature regarding cellular location, isolation and sample preparation for the respective antigenic target.

A General Guide To Sample Preparation: Use only a clarified solution without particles for the assay.

1. **Serum**: Coagulate at room temperature for 10-20 min. Centrifuge for 20 min at 2000-3000 rpm. Remove supernatant, If precipitation appears, centrifuge again. Assay the clarified supernatant fraction. A significant dilution of the sample (for example 1/100 – 1/1000) in the provided sample diluent or a buffer containing 1% BSA in PBS pH 7.4 is recommended for serum samples to enhance specificity due to the abundance of other

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proteins. Remember to take into account the dilution factor for sample quantitation.

2. **Plasma:** Use suitable EDTA or heparin as an anticoagulant. Mix for 10-20 min using a stir bar. Centrifuge for 20 min at 2000-3000 rpm. If precipitation appears, centrifuge again. Assay the clarified supernatant fraction. A significant dilution of the sample (for example 1/100 – 1/1000) in the provided sample diluent or a buffer containing 1% BSA in PBS pH 7.4 is recommended for plasma samples (as with serum samples) to enhance specificity due to the abundance of other proteins. Remember to take into account the dilution factor for sample quantitation.
3. **Urine**: Collect in a sterile container. Centrifuge 20-min at 2000-3000 rpm. Remove supernatant, If precipitation appears, centrifuge again. Assay supernatant.
4. **Cell culture supernatant:**. Detection of secretory components: Centrifuge culture media for 20 min at 2000-3000 rpm. Assay supernatant.
5. **Cell cytoplasm:** Dilute cell suspension with PBS (pH7.2-7.4) to a cell concentration of 1 million cells / ml. Perform repeated freeze-thaw cycles to fracture the cell membrane and to release intracellular components. Centrifuge for 20min at 2000-3000 rpm. Assay the supernatant, If precipitation appears, centrifuge again.
5. **Tissue:** Cut and weigh tissue slice. Add slice in PBS (pH7.2-7.4). Freeze rapidly with liquid nitrogen. Thaw sample to 2-8°C, add PBS and homogenize. Centrifuge for 20 min at 2000-3000rpm. Assay the clarified supernatant.
6. Samples can be store at -80°C. Avoid repeated freeze-thaw cycles. You may aliquot samples for later ELISA assays.
7. **Avoid denaturing cell lysis buffers that contain SDS such as RIPA buffer.**

Table 1. Materials Included with Kit. Store all materials at 4°C. The following is provided for the Human Monoamine Oxidase A ELISA kit. The research should adapt dilutions in a similar manner for other standard peptides and species.

1	Standard peptide (450ng/ml)	0.5ml		7	Chromogen Solution A	6ml
2	Standard diluent	1.5 ml		8	Chromogen Solution B	6ml
3	Microelisa Strip plate	12 well×8strips		9	Stop Solution	6ml
4	HRP-Conjugate Antibody	6 ml		10	Instruction Manual	1
5	30×wash solution	20ml		11	Sealed bags	1
6	Sample Diluent	6ml				

Materials required but not supplied

1. 37°C incubator
2. Standard absorbance microplate reader
3. Precision pipettes and disposable pipette tips
4. De-ionized water
5. Disposable tubes for sample dilution
6. Absorbent paper
7. 96 well dishes for preparation of solutions prior to transfer to the ELISA dish
8. 96-channel transfer pipette

Important notes and preparation for the assay: ***Inspect each solution for particles. If particles are present, centrifuge the solution and use the clarified supernatant.***

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1. The experimenter should perform preliminary tests to identify the sample dilutions that meet the assay range. Perform a preliminary assay with your samples, using the standard peptide at the low and high concentrations of the assay range of this kit. Suspend and dilute experimental samples into the “Sample Diluent (Table 1, Component 6)” to meet the assay range, (alternatively, dilute samples in PBS). A dilution series of several samples may be required to identify the correct sample concentration that meets the assay range. Concentrate or dilute experimental samples if adjustment is needed. Set aside sufficient experimental samples for reserve to repeat the assay.
2. Determine whether the vehicle buffer inadvertently cross-reacts with the assay and generates a color change by performing vehicle only controls. Additionally, determine whether ingredients in the vehicle buffer inhibit the assay reaction by diluting the provided standard peptide in the vehicle and perform an assay test. Compare results to the same standard peptide dilution in the Sample Diluent (Table 1, Component 6). For remedy, dilute samples in “Sample Diluent” (Table 1, Component 6) or prepare samples in another vehicle (such as 1% BSA in PBS pH 7.4) to prevent inadvertent experimental readings or assay inactivation.
3. The kit should be equilibrated to room temperate for 30 min prior to performing the assay. Store opened microelisa plates in a sealed plastic bag at 4°C. A multi-channel pipettor is the recommended method to simultaneously apply samples. Plates should be sealed during the assay. Wells should not be allowed to dry.
4. Perform preparations of standards and samples in separate tubes or 96-well plates, not in the ELISA plate wells. Transfer standards and samples simultaneously to the ELISA plate.
5. It is recommended that samples are assayed in duplicate to address pipetting error.
6. Use new applicator tips and ELISA plate sealants to avoid cross-contamination.
7. Do not mix reagents from other ELISA kits.
8. Note that sodium azide in samples that is not washed away may inhibit horse-radish peroxidase (HRP) that generates the color reaction of the assay.
9. When calculating concentration of your sample from the assay, make sure to take into account the dilution factor.
10. If the wash solution crystallizes during storage at 4°C, heat the solution at 37°C and shake until crystals suspend.

Assay procedures

Standard and Sample Preparation. Standards and Samples should be added simultaneously to wells**. Prepare the standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA dish.**

Assay procedure

1. Set aside and mark 10 wells for standard peptide dilutions. Configure five concentrations of standard peptide in duplicate as indicated in Table 2 below. Do not use the ELISA wells directly to perform the dilutions. The final total volume in each well should be 50µl.

Table 2. Standard Dilutions for human (Standard Peptide Concentration 450 ng/ml). Use similar dilution scheme for other species.

Well	Standard Concentration	Standard Number	Dilution Instructions
1	300 ng/ml	1	Mix 100µl Standard Peptide (Table 1, Component 1) with 50µl Standard Diluent (Table 1, Component 2). Remove 100µl to make standard 3.
2	300 ng/ml	2	Mix 100µl Standard Peptide with 50µl Standard Diluent. Remove 100µl to make standard 4.

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3	200 ng/ml	3	Mix 100µl Standard Number 1 with 50µl Standard Diluent. Remove 100µl to make standard 5.
4	200 ng/ml	4	Mix 100µl Standard Number 2 with 50µl Standard Diluent. Remove 100µl to make standard 6.
5	100 ng/ml	5	Mix 100µl Standard Number 3 with 100µl Standard Diluent. Remove 100µl to make standard 7. Remove 50µl, discard.
6	100 ng/ml	6	Mix 100µl Standard Number 4 with 100µl Standard Diluent. Remove 100µl to make standard 8. Remove 50µl, discard.
7	50 ng/ml	7	Mix 100µl Standard Number 5 with 100µl Standard Diluent. Remove 100µl to make standard 9. Remove 50µl, discard.
8	50 ng/ml	8	Mix 100µl Standard Number 6 with 100µl Standard Diluent. Remove 100µl to make standard 10. Remove 50µl, discard.
9	25 ng/ml	9	Mix 100µl Standard Number 7 with 100µl Standard Diluent. Remove 150µl, discard.
10	25 ng/ml	10	Mix 100µl Standard Number 8 with 100µl Standard Diluent. Remove 150µl, discard.

2. Set up 2 blank wells separately. In blank wells, add 40µl of the provided Sample Diluent (Component 6) and 10µl vehicle buffer that the sample is in; do not add sample. Perform all other procedures of the assay, except ***omit*** the HRP-conjugate antibody solution (Table 1, Component 4).

3. For wells with experimental samples, add 40µl provided Sample Diluent for each well, then add 10µl of experimental sample. (You may also use another dilution factor of sample). This creates a 5X dilution factor of sample which should be accounted for later when calculating sample concentration. The preparation should be done in separate tubes or 96-well plates, and not in the ELISA dish.

4. Transfer the standard peptide solutions, blank well solutions and diluted experimental samples simultaneously to the ELISA dish. Use the closure membrane to enclose the plate, mix gently with a rotator table, and incubate for 30 min at 37°C, or 1 hr at RT.

5. Dilute the 30X Wash Solution (Table 1, Component 5) with dH_20. Make 3 ml of diluted wash solution for each assay well.

6. After the first incubation period, discard the liquid in the wells by gently aspirating. Turn the plate upside down and gently pat dry the plate with absorbent paper. To wash the wells, simultaneously fill each well with 100 µl of diluted Wash Solution, oscillate gently with the rotator table for 1.5 min, and then aspirate off the liquid. Pat dry the ELISA microplate with absorbent paper. Repeat the wash steps 5 more times with 30 seconds per wash instead of 1.5 min.

7. Prepare in a separate 96-well dish 50 µl HRP-conjugate antibody reagent (Table 1, Component 4) for each well, *except* the blank wells. Simultaneously transfer 50 µl HRP-conjugate antibody reagent to each well, except for the blank wells. Rotate gently for 1 hr at 37°C for 30 min or 1 hr at RT.

8. Wash the wells 5X as described above in step 6. Remove the liquid from the wells. Do not allow wells to dry.

9. To each well, dispense 50 µl Chromogen Solution A (Table 1, Component 7) and then dispense 50µl of

Chromogen Solution B (Table 1, Component 8) for each well. Mix the ELISA plate gently for 15 min at 37°C in the dark. Chromogen solution B is light sensitive.

10. Simultaneously add 50 µl of Stop Solution (Table 1, Component 9) to each well. Upon addition of stop solution, the blue color should immediately change to yellow.

11. Measure the optimal density (OD) at 450 nm within 15 minutes after adding stop solution. Set the blank wells as zero.

Data Analysis

1. Compile a standard curve using the blank standard solutions and corresponding OD values. You may wish to calculate a linear regression equation to determine the concentration of your samples. Account for the dilution factors in your final calculation. Other data analysis methods to calculate sample concentrations are also applicable.

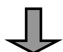
Flow chart of the procedures

Prepare standards, blank and samples

Add samples to wells, incubate for 1 hr at RT or 30 min at 37°C.

Wash each well five times.

Add HRP-conjugate antibody to each well, incubate for 1 hr at RT or 30 min at 37°C.



Wash each well five times

Add chromogen solutions A and B, 15 min at 37°C, dark

Add stop solution

Measure OD 450 nm within 15 min

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