Version 22-04/24

User's Manual

Vanillyl mandelic acid (VMA) ELISA Kit

REF **DEIA2255**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

General Description

Vanillyl mandelic acid (VMA) is an end-stage metabolite of the catecholamines epinephrine and norepinephrine. It is produced via intermediary metabolites. VMA is found in the urine, along with other catecholamine metabolites, including homovanillic acid (HVA), metanephrine and normetanephrine. In timed urine tests the quantity excreted (usually per 24 hours) is assessed, along with creatinine clearance, and the quantity of cortisols, catecholamines, and metanephrines excreted.

Principles of Testing

Vanillyl mandelic acid (VMA) ELISA Kit is based on a competitive enzyme immunoassay technique. The microtiter well-plate in this kit has been pre-coated with an anti-VMA antibody. Sample or standards are added to the wells along with a fixed quantity of biotinylated VMA and incubated. The VMA found in the sample or standards competes with the biotinylated VMA for limited binding sites on the immobilized anti-VMA antibody. Excess unbound biotinylated VMA and sample or standard VMA is washed from the plate. Avidin-HRP conjugate is added, incubated and washed. An enzymatic reaction is then produced through the addition of TMB substrate which is catalyzed by the immobilized HRP to generate a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is measured by reading the absorbance at 450 nm which is quantitatively proportional to the amount of biotinylated VMA captured in the well and inversely proportional to the amount of VMA which was contained in the sample or standard.

Reagents And Materials Provided

Store at -20°C for 6 months:

1. VMA Microplate, 96 Wells (12 x 8 Well strips)
2. VMA Lyophilized Standard, 2 vials
3. 100X VMA-Biotin Complex, 1 x 60 µL
4. 100X Avidin-HRP Conjugate, 1 x 120 µL
5. Sample Diluent, 1 x 20 mL
6. Biotin Complex Diluent, 1 x 12 mL
7. Conjugate Diluent, 1 x 12 mL
8. 25X Wash Buffer, 1 x 30 mL

Store at 4°C for 6 months:

1. Stop Solution, 1 x 10 mL
2. TMB Substrate, 1 x 10 mL

Materials Required But Not Supplied

1. Microplate reader capable of reading absorbance at 450 nm.
2. Automated plate washer (optional).

3. Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
4. Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
5. New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
6. Absorbent paper or paper toweling.
7. Distilled or deionized ultrapure water.
8. 37°C Incubator (optional).

Storage

Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

Specimen Collection And Preparation

1. Sample Storage

1.1 Samples must be tested to determine if the kit is valid.

1.2 Store samples to be assayed at 4°C for 24 hours prior being assayed.

1.3 For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

2. General Sample Preparation Guidelines:

2.1 **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

2.2 **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

2.3 **Tissue Homogenates** – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenate for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

2.4 **Cell Lysates** - Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1×) and ultrasonicate the cells 4 times (or freeze cells at ≤ -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.) Centrifuge at 1,500 x g for 10 minutes at 2 - 8°C to remove cellular debris.

2.5 **Cell culture supernatants and other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

3. Recombinant Proteins: Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

4. Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

4.1 Prior to performing the full experiment, test a serially diluted representative sample. Alternately, a small pool of several samples can also be used with this same method if sample volume is limited or refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit.

4.2 Dilute samples using **Sample Diluent**.

4.3 Mix diluted samples gently and thoroughly.

4.4 Pipetting less than 2 µL is not recommended for optimal assay accuracy.

Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

1. Standards

1.1 Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. For further directions, please refer to the Certificate of Analysis.

2. 1X VMA-Biotin Complex

2.1 Prepare the **1X VMA-Biotin Complex** immediately prior to use by diluting the **100X VMA-Biotin Complex** 1:100 with **Complex Diluent**.

2.2 For each well strip to be used in the experiment (8-wells) prepare 500 µL by adding 5 µL of **100X VMA-Biotin Complex** to 495 µL **Complex Diluent**.

2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

3. 1X Avidin-HRP Conjugate

3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X AvidinHRP Conjugate** 1:100 with **Conjugate Diluent** as follows.

3.2 Briefly and gently mix the **100X Avidin-HRP Conjugate** prior to pipetting.

3.3 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL **1X Avidin-HRP Conjugate** by adding 10 µL of **100X Avidin-HRP Conjugate to 990 µL Conjugate Diluent**.

3.4 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

4. Microplate Preparation

4.1 Micro-plates are provided ready to use and do not require rinsing or blocking.

4.2 Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

4.3 Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

5. 1X Wash Buffer

5.1 If crystals have formed in the **25X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

5.2 Add the entire 30 mL contents of the **25X Wash Buffer** bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

5.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

5.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer**at 4°C for no longer than 1 week. Do not freeze.

Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

1. Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.
2. Retain at least one well as an absolute Blank without any samples or reagents.
3. Add 50 µL of serially titrated standards, diluted samples or blank into wells of the **VMA Microplate**. At least two replicates of each standard, sample or blank is recommended.
4. Immediately add 50 µL of **1X VMA-Biotin Complex** to each well (excluding absolute Blank).
5. Cover the plate with the plate sealer and incubate for 60 minutes.
6. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
7. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
8. Wash plate three times with **1X Wash Buffer** as follows:

   8.1 Add 200 µL of **1X Wash Buffer** to each assay well.

   8.2 Incubate for 2 minutes.

   8.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

   8.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

   8.5 Repeat steps 8.1 through 8.4 **two** more times.
9. Add 100 µL of **1X Avidin-HRP Conjugate** to each well.
10. Cover the plate with the plate sealer and incubate for 45 minutes.
11. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
12. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
13. Repeat wash as in **step 8**.
14. Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep based on the standard, adjust incubation times.

    (NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized

by blue shading in the bottom four standard wells, while the remaining standards still appear clearer.)

15. Add 50µL of **Stop Solution** to each well. Well color should change to gradations of yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.
16. Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 15. If wavelength correction is available, set to 540 nm or 570 nm.

Calculation

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

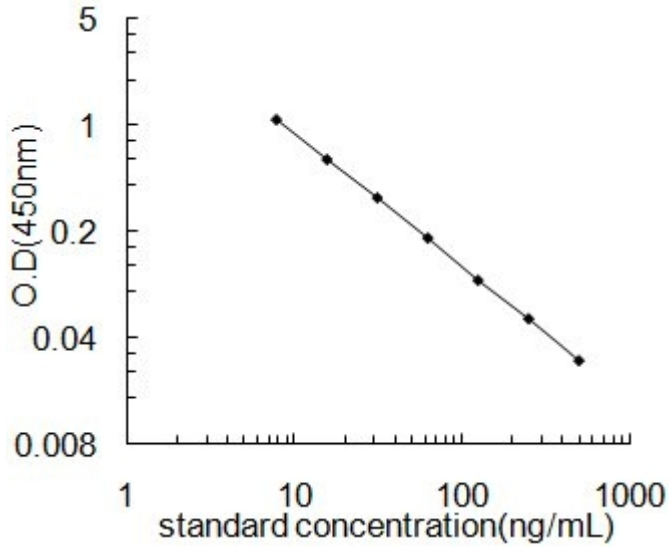
(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

Typical Standard Curve



Detection Range

7.8 - 500ng/mL

Sensitivity

2.3 ng/mL

Specificity

Natural and recombinant General Vanillyl mandelic acid

Recovery

Mean recovery when spiking into Serum and Plasma = 109%

Reproducibility

Mean Intra-assay CV%: < 5.2% (n = 20)

Mean Inter-assay CV%: < 8.6% (n = 20)

Precautions

1. Read instructions fully prior to beginning use of the assay kit.
2. Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
3. Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
4. For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
5. Kit cannot be used beyond the expiration date on the label.