Version 22-04/24

User's Manual

ADMA Xpress ELISA Kit

REF **DEIA080J**

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

Intended Use

This ELISA Kit is intended for the quantitative determination of asymmetric dimethyl arginine (ADMA) in serum, citrate and EDTA plasma. For research use only. Not for use in diagnostic procedures.

Principles of Testing

This ELISA is designed for the quantitative determination of ADMA.This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of a derivatisation reagent for ADMA derivatisation, and a reaction buffer is added containing ADMA-derivative (tracer). Afterwards, the treated samples are incubated in wells of a microtiter plate coated with a polyclonal antibody against ADMA-derivative. During the incubation period the target ADMA in the sample competes with the tracer for the binding of the polyclonal antibodies, immobilised on the wall of the microtiter wells. During the second incubation step a peroxidase conjugate is added to each microtiter well to detect the tracer. After washing away the unbound components, tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow, and the absorbance is measured in a photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the ADMA concentration in the sample; this means, high ADMA concentration in the sample reduces the concentration of antibody-bound tracer and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. ADMA, present in the samples, is determined directly from this curve.

Reagents And Materials Provided

1. PLATE, Microtiter plate, pre-coated, 12×8wells 2. STD, Standards,ready-to-use (0, 0.1, 0.2, 0.4, 0.8, 2.0µM), 6×200µl 3. CTRL 1, Control, ready-to-use, 1×200µl 4. CTRL 2, Control, ready-to-use, 1×200µl 5. WASHBUFA, 10× Wash buffer concentrate, 2×100 ml 6. REABUF, Reaction buffer, ready-to-use, 1×22ml 7. CONJ, Conjugate, ready-to-use, 1×12ml 8. DER, Derivatisation reagent, lyophilised, 1 vial 9. DMSO, Dimethylsulfoxide (DMSO), 1×4ml 10. CODIL, Dilution buffer after derivatization, ready-to-use, 1×18 ml 11. SUB, Substrate (tetramethylbenzidine),ready-to-use, 1×15ml 12. STOP, Stop solution, ready-to-use, 1×15 ml

Materials Required But Not Supplied

1. Ultrapure water* 2. Calibrated precision pipets and 10-1000 µl single-use tips 3. Foil to cover the microtiter plate 4. Horizontal microtiter plate shaker 5. Multi-channel pipets or repeater pipets 6. Vortex 7. Standard single-use laboratory glass or plastic vials, cups, etc. 8. Microtiter plate reader CD recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules(free of particles > 0.2 um) with an electrical conductivity of 0.055 µuS/cm at 25 °C(≥18.2MΩcm).

Storage

2-8°C, Please store the Standards and two Controls at -20°C. Avoid repeated freezing and thawing.

Specimen Collection And Preparation

Sample: Serum, citrate and EDTA plasma 1. Freshly collected serum or plasma can be stored for 3 days at room temperature (15-30°C) or at 2-8°C. For longer storage keep frozen at -20°C. 2. Lipemic or hemolytic samples may give erroneous results and should not be used for analysis. 3. EDTA plasma and serum samples are used **undiluted**. If sample dilution is required, use STD1 (zero-standard) as diluent. 4. For sample preparation, a derivatisation reagent for derivatisation of ADMA is added (details are given in the sample preparation procedure).

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. 2. Preparation of the wash buffer: The wash buffer concentrate (WASHBUF A) has to be diluted with ultrapure water 1:10 before use,(100 ml WASHBUF A + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37°C. The WASHBUF A is stable at 2-8°C until the expiry date stated on the label. Wash buffer (1:10 diluted WASHBUF A) can be stored in a closed flask at 2-8°Cfor 1 month. 3. Store standards and controls (STD/CTRL) frozen at -20°C. They are stable at -20°C until theexpiry date stated on the label. Thaw before use in the test and mix well. Re-freeze standards and controls after use. 4. The reaction buffer (REABUF) is stable at 2-8°C until the expiry date stated on the label. Bring to room temperature before use and dissolve any occurring crystals. 5. DMSO crystallises at 2-8°C. Before use, bring to room temperature to dissolve the crystals. 6. The lyophilisedderivatisation reagent (DER) is stable at2-8°Cuntil the expiry date stated on the label. Bring to room temperature before opening and reconstitute the content of the vial with 3 ml DMS0. Mix thoroughly with a vortex-mixer and allow to dissolve for 10 minutes. The derivatisation reagent (reconstituted DER) can be stored at 2-8°C for 2 months. Bring to room temperature before reuse. **Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.** 7. All other test reagents are ready-to-use. Test reagents are stable until the ring all reagents and samples to room temperature (15-30°C) and mix well.expiry date(see label) when stored at 2-8°C.

Assay Procedure

Bring all reagents and samples to room temperature(15-30°C) and mix well. Derivatisation of standards, controls and samples is carried out in single analysis in vials (e.g. 1.5 ml polypropylene vials). We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate. 1. Add 25µl standard (STD)/control (CTRL)/sample into the corresponding vials. 2. Add 200µl reaction buffer(REABUF) into each vial (STD, CTRL, sample). 3. Add 25µl derivatisation reagent into each vial (STD, CTRL, sample) and mix thoroughly by repeated inversion or several seconds on a vortex mixer. Incubate for 30min at room temperature (15-30°C) on a horizontal shaker. **2×50µl of the derivatised standards/controls/samples are used in the ELISA as duplicates.** Mark the positions of standards/controls/samples in duplicate on a protocol sheet. Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2-8°C. Strips are stable until the expiry date stated on the label. 4. Before use, wash the wells 5 times with 250µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on

absorbent paper. 5. Add 150µl dilution buffer (CODIL) into each well of the microtiter plate. 6. For the analysis in duplicate take 2×50µl of the derivatised standards/controls/samples out of the vials and add into the respective wells of the microtiter plate. 7. Cover the strips and incubate for 2 hours at room temperature(15-30°C) on a horizontal shaker. 8. Discard the content of each well and wash 5 times with 250 µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper. 9. Add 100µl conjugate (CONJ) into each well. 10. Cover the strips and incubate for 30 minutes at room temperature (15-30°C) on a horizontal shaker. 11. Discard the content of each well and wash 5 timeswith 250µl wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper. 12. Add 100µl substrate (SUB) into each well. 13. Incubate for 8-12 min at room temperature(15-30°C) in the dark. (**Note: The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation.**) 14. Add 100µl stop solution(STOP) into each well and mix well. 15. Determine absorption immediately with an ELISA reader at 450nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620nm (690 nm) as a reference.

Quality Control

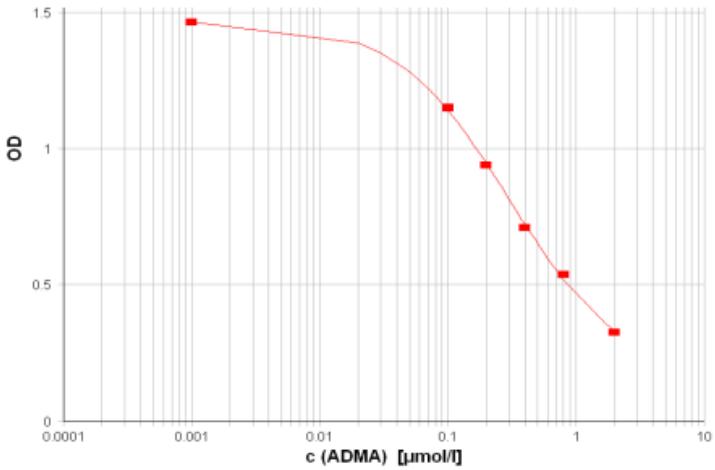
CD recommends the use of external controls for internal quality control, if possible. Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control samples are outside of the acceptable limits. We recommend each laboratory to establish its own reference range.

Calculation

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm. **1. 4 parameter algorithm** It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1(e.g. 0.001). **2. Point-to-point calculation** We recommend a linear ordinate for optical density and a linear abscissa for concentration. **3. Spline algorithm** We recommend a linear ordinate for optical density and a linear abscissa for concentration. The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually. **Serum, citrate and EDTA plasma No factor** is required.

Typical Standard Curve

In the following, an example of a standard curve is given. Do not use it for the calculation of your results.



Precision

Intra-assay (n= 12)

Sample	ADMA [µmol/l]	CV [%]
1	0.19	7.9
2	0.48	5.8

Inter-assay (n= 12)

Sample	ADMA [µmol/l]	CV [%]
1	0.19	10.8
2	0.47	7.6

Detection Limit

Samples with concentrations above the measurement range can be diluted with Standard 1 (zero-standard) and re-assayed. Please consider this dilution factor when calculating the results. Samples with concentrations lower than the measurement range cannot be clearly quantified. The upper limit of the measurement range can be calculated as: highest concentration of the standard curve × sample dilution factor to be used The lower limit of the measurement range can be calculated as: Analytical sensitivity × sample dilution factor to be used

Sensitivity

The zero-standard (STD 1) was measured 46 times. The detection limit was set as B_0- 2 SD and estimated to be 0.04 umol/l.

Interferences

Biotin interference Samples containing a biotin concentration of ≤ 1200 ng/ml show a change of the results of < 25 %. Higher concentrations of biotin can lead to falsely low results.

Precautions

1. The guidelines for laboratories should be followed. 2. Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. CD can therefore not be held responsible for any damage resulting from incorrect use. 3. Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to CD along with a written complaint. 4. All reagents in the kit package are for research use only. 5. Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious. 6. Kit reagents contain thimerosal or ProClin as bactericides. Thimerosal and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes. 7. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

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