Tricat (Adrenalin, Noradrenalin, Dopamine) ELISA Kit

Cat. No.:DEIA2011
Pkg.Size:96T

Intended use
Manual and automated enzyme immunoassay for measurement of adrenalin (epinephrine), noradrenalin (norepinephrine) and dopamine in human plasma and urine.

Principle Of The Test
Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with a goat anti rabbit antibody. The added liquid antibody, directed towards an epitope of an antigen molecule binds to the plate within the incubation time. The antigen of the sample is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After the substrate reaction the intensity of the developed color is proportional to the amount of the antigen. Results of samples can be determined directly using the standard curve.

Reagents And Materials Provided
The reagents provided with this kit are sufficient for 96 extractions in single determinations in the sample preparation (extraction): 88 specimen samples, 6 standards and 2 controls. Each extract is sufficient for a single determination for adrenalin, noradrenalin and dopamine immunoassay.

1. **Microtiter Plate:** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal). 3 x 12 x 8
2. **Standard A-F:**
   - Adrenalin: 0; 1.5; 5.0; 15; 50; 150 ng/mL (0; 8; 27; 82; 273; 819 nmol/L)
   - Noradrenalin: 0; 5.0; 15; 50; 150; 500 ng/mL (0; 30; 89; 296; 887; 2955 nmol/L)
   - Dopamine: 0; 60; 180; 585; 2300; 11470 ng/mL (0; 392; 1175; 3819; 15014; 74876 nmol/L)
   Ready to use. Contains: [-] Adrenalin, [-] Noradrenalin, [-] Dopamine (biologically active), and 0.1 M HCl. 1 x 6 x 2.5 mL
3. **Control 1+2:**
   Ready to use. Contains: [-] Adrenalin, [-] Noradrenalin, [-] Dopamine (biologically active), 0.1 M HCl. Exact concentrations see vial labels or QC certificate. 1 x 2 x 2.5 mL
4. **Enzyme Conjugate Concentrate (100x):** Contains: antibodies, conjugated to alkaline phosphatase, Tris buffer, HCl, 0.01 % NaN3. 2 x 250 µL
5. **Extraction Plate(Macrotiter Plate):** 24 wells each. Coated with boronate affinity gel. 4 x
6. **Extraction Buffer:** Pink colored. Ready to use. Contains: 0.016 % NaN3. 2 x 60 mL
7. **COMT lyophilized:** Contains: Catechol-O-methyltransferase (porcine liver), NaN3. 6 x 1.25 mL
8. **Coenzyme Solution Ready to use.** Contains: S-Adenosyl-L-Methionine, stabilizers. 6 x 1.25 mL
9. **Enzyme Buffer:** Ready to use. Contains: Tris buffer, HCl, stabilizers. 3 x 3 mL
10. **Release Buffer:** Yellow Colored. Ready to use. Contains: 0.1 M HCl, indicator. 1 x 100 mL
12. **Wash Buffer Concentrate (10x):** Contains: Tris buffer, HCl, Tween, 0.2 % NaN3. 3 x 50 mL
13. **COMT Additive:** Contains: human plasma, stabilizers, 0.01 % Thimerosal. 2 x 2 mL
14. **Adrenalin Antiserum:** Green colored. Ready to use. Contains: antibodies against Adrenalin (rabbit), Buffer, stabilizers. 1 x 7.0 mL
15. **Noradrenalin Antiserum:** Blue colored. Ready to use. Contains: antibodies against Noradrenalin (rabbit), Buffer, stabilizers. 1
Materials Required But Not Supplied

1. Micropipettes (Micropette Eppendorf or similar devices, < 3% CV). Volume: 10; 10-100; 100-1000 µL
2. Orbital shaker (200-900 rpm)
3. Vortex mixer
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer
9. Disposable tubes for sample dilution
10. 0.1 M HCl, for sample dilution (Urine)

Storage

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

Specimen Collection And Handling

NOTE: The in-vivo catecholamine and metanephrines release is influenced by several foods and drugs. Vitamin B, coffee and bananas, alpha-methyldopa, MAO and COMT inhibitors as well as medications related to hypertension should be discontinued for at least 72 h prior to specimen collection.

Plasma (EDTA)
The blood sample should be stored at 2-8°C until centrifuged to separate the plasma within 2 h after blood collection. The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Urine
It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle containing 10-15 mL of 6 N HCl as preservative. Determine total volume for calculation of results. Mix and centrifuge samples before use in the assay.

Reagent Preparation

The volumes stated below are for one run with 3 x 6 strips (3 x 48 determinations)
Assay Steps

Dilution of Samples

Samples suspected to contain concentrations above the highest standard have to be diluted as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 mL Wash Buffer Concentrate</td>
<td>575 mL bidist. Water</td>
<td>1:10</td>
<td>Mix vigorously</td>
<td>2-8°C</td>
<td>4 w</td>
</tr>
<tr>
<td>150 µL Enzyme Conjugate</td>
<td>15 mL 1x Wash Solution</td>
<td>1:100</td>
<td>Prepare freshly and use only once. Mix without foaming.</td>
<td>18-25°C</td>
<td>5 h</td>
</tr>
<tr>
<td>12 PNPP Substrate Tablets</td>
<td>32 mL PNPP Substrate Buffer</td>
<td></td>
<td></td>
<td>18-25°C</td>
<td>5 h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>to be diluted</th>
<th>with</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>&gt; highest standard</td>
<td>bidist. water</td>
<td>prior to extraction step</td>
</tr>
<tr>
<td>Urine</td>
<td>&gt; highest standard</td>
<td>0.1 N HCl</td>
<td>prior to extraction step</td>
</tr>
</tbody>
</table>

Extraction of Samples, Standards and Controls (Extraction Plate) (manual version)

1. Pipette 20 µL of each Standard, Control and urine sample and 500 µL of each plasma sample into the respective wells of the extraction plate. Add 500 µL of bidist. water to all wells except for the plasma samples to correct differences of volumes.
2. Pipette 1000 µL of Extraction Buffer into each well.
3. Cover plate with adhesive foil. Extract 30 min at RT (18-25°C) on an orbital shaker (600-900 rpm).
During extraction the surface of the liquid should wet the adhesive foil, but the liquid level should not exceed 2/3 of the well. Splashing does not affect results.
4. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel.
5. Pipette 2 mL of bidist water into each well.
6. Cover plate with new adhesive foil.
Shake 5 min at RT (18-25°C) on an orbital shaker (600-900 rpm). Splashing does not affect results.
8. Pipette 150 µL of Extraction Buffer into each well. To each well add 50 µL of Acylation Reagent. Mix immediately after pipetting.
9. Extract 20 min at RT (18-25°C) (without adhesive foil) on an orbital shaker (400-600 rpm).
10. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
11. Pipette 2 mL of bidist. water into each well.
12. Cover plate with new adhesive foil. Shake 5 min at RT (18-25°C) on an orbital shaker (600-900 rpm). Splashing does not affect results.
14. Pipette 300 µL of Release Buffer into each well.
15. Shake 30 min at RT (18-25°C) (without adhesive foil) on an orbital shaker (400-600 rpm).
Prepared samples should be assayed the same day. If this is not possible, you can store the extraction plate covered with adhesive foil at 2-8°C over night. Important for measurement of Dopamine

Dilution of extracted standard, controls must be performed prior to pipetting into wells of Microtiter plate in extra tubes. Predilute urine samples well.
Therefore, dilute all extracted Standards, Controls and urine samples 1:51 with Release Buffer in disposable tubes. (i.e. 10 µL extracted samples + 500 µL Release Buffer).
Extracted plasma samples do not require this predilution.

Preparation of COMT Enzyme Solution
The COMT Enzyme Solution should be freshly prepared directly before use.

Dissolve each kit component of lyophilized COMT in 1.25 mL bidist. water and mix the dissolved COMT.*

Then pipette 1.25 mL of Coenzyme Solution followed by 1.25 mL of Enzyme Buffer and 0.40 mL COMT Additiveto the mixed COMT vials to give a final volume of 4.15 mL of COMT Enzyme Solution per vial.

Pool three vials for 48 determinations of adrenalin and 48 determinations of noradrenalin and 48 determinations of dopamine. Solution may be turbid. Mix without foaming. The COMT solution is stable at room temperature for 1 hour.

* If only an aliquot of the COMT solution is needed, the rest of the COMT solution should be frozen immediately in aliquots at -20°C. The COMT solution is stable under these conditions for 1-2 months.

**Enzymatic Derivatization of Samples, Standards and Controls (Microtiter Plate)**

If pipetting with positive displacement, give the residual fluid from the tip of the pipette back to the corresponding wells of the extraction plate, otherwise the extracts may not be sufficient for the determination of the other analytes.

It is useful to hold the extraction plate in a sloping position.

Before use of the Microtiter plates, define and label the wells for Adrenalin, Noradrenalin and Dopamine.

For research use of tissue homogenates and cell culture supernatants a general recommendation can be given:

Working with cell culture supernatants depends on the matrix as well as concentrations expected:

According to the urine protocol (extraction of at least 20 µL supernatant) a sensitivity for adrenalin of 0.3 ng/mL, for noradrenaline of 0.6 ng/mL and for dopamine of 5 ng/mL for diluted sample can be expected. In case of a matrix with addition of serum (FCS), plasma protocol (extraction of 500 µL supernatant) can be used with the sensitivities corresponding to the plasma protocol.

For tissue homogenates no perchloric acid should be used for homogenization. For further details ask CD.

**Adrenaline for urine and plasma**

1. Pipette 75 µL of freshly prepared COMT Enzyme Solution into each well of the Microtiter Plate. Shake plate briefly.
2. Pipette 100 µL of each extracted Standard, Control and sample into the respective wells. During this step hold the pipette tips directly into the COMT solution. Color changes to pink. Shake briefly.
3. Pipette 50 µL of Adrenalin Antiserum (green colored) into each well.
4. Cover plate with adhesive foil. Incubate 120 min at RT (18-25°C) on an orbital shaker (400-600 rpm).

**Noradrenaline for urine and plasma**

1. Pipette 25 µL of freshly prepared COMT Enzyme Solution into each well of the Microtiter Plate. Shake plate briefly.
2. Pipette 25 µL of each extracted Standard, Control and sample into the respective wells. During this step hold the pipette tips directly into the COMT solution. Color changes to pink. Shake plate briefly.
3. Pipette 50 µL of Noradrenalin Antiserum (blue colored) into each well.
4. Cover plate with adhesive foil. Incubate 120 min at RT (18-25°C) on an orbital shaker (400-600 rpm).

**Dopamine for urine and plasma**

1. Pipette 75 µL of freshly prepared COMT Enzyme Solution into each well of the Microtiter Plate. Shake briefly.
2. For urine: Pipette 100 µL of 1:51 prediluted extracted Standard, Control and urine sample into the respective wells. During this step hold the pipette tips directly into the COMT solution. Color changes to pink. Shake briefly.
3. For plasma: Pipette 100 µL of 1:51 prediluted extracted Standard, Control and undiluted extracted plasma sample into the respective wells. During this step hold the pipette tips directly into the COMT solution. Color changes to pink. Shake briefly.
4. Pipette 50 µL of Dopamine Antiserum (violet colored) into each well.
5. Cover plate with adhesive foil. Incubate 120 min at RT (18-25°C) on an orbital shaker (400–600 rpm).

**ELISA**

The following procedure must be performed for Adrenalin, Noradrenalin and Dopamine.

1. Remove adhesive foil. Discard incubation solution. Wash plate 6 x with 250-300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
2. Pipette 100 µL of freshly prepared Enzyme Conjugate into each well.
3. Cover plate with new adhesive foil. Incubate 60 min at RT (18-25°C) on an orbital shaker (400-600 rpm).
4. Remove adhesive foil. Discard incubation solution. Wash plate 6 x with 250-300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
5. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6. Pipette 200 µL of PNPP Substrate Solution into each well.
7. Incubate 40 min at RT (18-25°C) (without adhesive foil) on an orbital shaker (400–600 rpm).
8. Stop the substrate reaction by adding 50 µL of PNPP Stop Solution into each well. Briefly mix contents by gently shaking the plate.
9. Measure optical density with a photometer at 405 nm (Reference-wavelength: 620-650 nm) within 60 min after pipetting of the Stop Solution. No air bubbles should be visible.

**Quality Control**

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

**Calculation**

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards.

The concentrations for adrenaline, noradrenaline and dopamine of the kit Controls and of the urine samples in ng/mL can be read directly from the corresponding standard curve.

The results for Adrenaline and Noradrenaline plasma samples in ng/mL have to be divided by 25. This correction factor responds to the difference in the volume required during the extraction step (20 µL of standards vs 500 µL plasma for manual version and 30 µL standards vs 750 µL plasma for the automated version).

The results for Dopamine plasma samples have to be divided by 1275. This correction factor responds to the difference above mentioned during the extraction procedure and to the 1:51 predilution of the standards. To convert from ng/mL to pg/mL please multiply by 1000.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above the highest standard have to be diluted and rea

**Conversion:**

1000 pg/mL = 1 ng/mL

Adrenalin (µg/L) x 5.458 = nmol/L
Noradrenalin (µg/L) x 5.911 = nmol/L
Dopamine (µg/L) x 6.528 = nmol/L ssayed.

Calculate the 24 h excretion for each urine sample: µg/24h = µg/L x L/24h

**Typical Standard Curve**

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.
Precautions

1. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
2. In case of severe damage of the kit package please contact or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. Material Safety Data Sheets for this product are available upon request.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
7. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg
and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

REFERENCES