Human Adrenaline ELISA Kit

Enzyme Immunoassay for the quantification of Adrenaline in human plasma and urine samples.

Catalog number: ARG80442

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Epinephrine (also known as adrenaline, adrenalin, or β,3,4-trihydroxy-N-methylphenethylamine) is a hormone and a neurotransmitter. Epinephrine is indicated for intravenous injection in treatment of acute hypersensitivity, treatment of acute asthmatic attacks to relieve bronchospasm, and treatment and prophylaxis of cardiac arrest and attacks of transitory atrioventricular heart block with syncopal seizures (Stokes-Adams Syndrome). The actions of epinephrine resemble the effects of stimulation of adrenergic nerves. To a variable degree it acts on both alpha and beta receptor sites of sympathetic effector cells. Its most prominent actions are on the beta receptors of the heart, vascular and other smooth muscle. When given by rapid intravenous injection, it produces a rapid rise in blood pressure, mainly systolic, by (1) direct stimulation of cardiac muscle which increases the strength of ventricular contraction, (2) increasing the heart rate and (3) constriction of the arterioles in the skin, mucosa and splanchnic areas of the circulation. When given by slow intravenous injection, epinephrine usually produces only a moderate rise in systolic and a fall in diastolic pressure. Although some increase in pulse pressure occurs, there is usually no great elevation in mean blood pressure. Accordingly, the compensatory reflex mechanisms that come into play with a pronounced increase in blood pressure do not antagonize the direct cardiac actions of epinephrine as much as with catecholamines that have a predominant action on alpha receptors. [Provide by Drugbank]

PRINCIPLE OF THE ASSAY

This is a competitive enzyme Immunoassay for the quantification of Adrenaline (Epinephrine) in plasma and urine samples.

This assay employs the competitive quantitative enzyme immunoassay technique. First, Adrenaline is extracted by using a cis-diol specific affinity gel, acylated and then derivatized enzymatically. The antigen has been pre-coated onto a microtiter plate. Derivatized controls, standards or samples and the solid phase bound analytes compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. Anti-rabbit IgG conjugated to Peroxidase is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Adrenaline present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Adrenaline in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Adrenaline Microtiter Strips	12 strips X 8 wells	4°C
Extraction Plate (coated with boronate affinity gel)	2 X 48 wells	4°C
Adhesive foil	1 X 4 pieces	RT
50X Wash Buffer	1 X 20ml	4°C
Anti-rabbit IgG-peroxidase conjugate	1 X 12ml (Ready-to-use)	4°C
TMB substrate	1 X 12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	1 X 12ml (Ready-to-use)	4°C
Adjustment Buffer	4ml (Ready-to-use)	4°C
Standard A-F (0, 1, 4, 15, 50, 200 ng/ml)	4ml each (Ready-to-use)	4°C
Adrenaline Antiserum	6ml (Ready-to-use)	4°C
Assay Buffer	6ml (Ready-to-use)	4°C
Control 1 (8 ng/ml ± 40%)	4ml (Ready-to-use)	4°C
Control 2 (30 ng/ml ± 40%)	4ml (Ready-to-use)	4°C
Acylation Buffer	20ml (Ready-to-use)	4°C
Acylation Reagent	3ml (Ready-to-use)	4°C
Coenzyme (S-adenosyl-L-methionine)	4ml (Ready-to-use)	4°C
Enzyme (COMT)	2 X 1ml (Lyophilized)	4°C
Extraction Buffer	6ml (Ready-to-use)	4°C
Hydrochloric Acid	1 X 20ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as reference wavelength)
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.
- Store the unopened reagents at 2 -8°C until expiration date. Once opened the reagents are stable for 1 month when stored at 2-8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

SAMPLE COLLECTION & STORAGE INFORMATION

Urine – Spontaneous or 24-hour urine, collected in a bottle containing 10-15ml of 6M HCl. If 24-hour urine is used, please record the total volume of the collected urine. Store at 2-8 °C up to 48 hours, or store at room temperature up to 24 hours. For longer storage, aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

Plasma - Collect plasma using EDTA as an anticoagulant. Do not use haemolytic or lipemic samples. Assay immediately (up to 6 hours at 2-8 °C), or aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 1 months at 2-8°C.
- Enzyme Solution: Reconstitute the lyophilized "Enzyme (COMT)" with 1ml of distilled water and mix well. Then add 0.3ml coenzyme followed by 0.7ml Adjustment buffer. The total volume of Enzyme solution is 2ml. Prepare fresh prior to assay (not more than 10-15 minutes in advance). Discard unused Enzyme solution.

ASSAY PROCEDURE

Sample Preparation, Extraction and Acylation

1. Pipette 10µl of standards, controls, urine samples and 300µl of plasma samples into the appropriate wells of the Extraction Plate. (note: The read concentrations of plasma samples have to be divided by 30)
2. Add 250µl of distilled water to wells with standards, controls and urine samples.
3. Add 50µl Assay Buffer to all wells.
4. Add 50µl Extraction Buffer into all wells.
5. Cover plate and incubate for 30 mins at RT on a microplate shaker (~600rpm).
6. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1ml wash buffer and shake for 5 mins at RT on a microplate shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.
7. Repeat wash as step 6. Discard and blot dry by tapping the inverted plate on absorbent material.
8. Add 150µl Acylation Buffer into all wells.
9. Add 25µl Acylation Reagent into all wells.
10. Incubate for 15 mins at RT on a microplate shaker (~600rpm)
11. Remove foil, discard and blot dry by tapping the inverted plate on absorbent material. Wash each well with 1ml wash buffer and shake for 10 mins at RT on a microplate shaker (600rpm). Blot dry by tapping the inverted plate on absorbent material.

12. Add 150µl Hydrochloric Acid into all wells.
13. Cover plate and incubate for 10 mins at RT on a microplate shaker (~600rpm)
14. Remove foil, **do not decant the supernatant!** 100 µl of the supernatant is needed for the subsequent Adrenaline ELISA procedure.

Adrenaline ELISA procedure

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
1. Add 25 µl Enzyme solution (please refer to REAGENT PREPARATION section) into all wells of the Adrenaline Microtiter Strips.
2. Add 100 µl extracted standards, controls, and samples into appropriate wells of the Adrenaline Microtiter Strips.
3. Incubate 30 mins at RT on a microplate shaker (600rpm).
4. Add 50 µl of Adrenaline Antiserum into all wells.
5. Cover plate with foil, incubate for 2 hours at RT on a microplate shaker (600rpm).
6. Remove the foil and discard. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add 100 µl of Anti-rabbit IgG-peroxidase conjugate into wells.
8. Incubate for 30 mins at RT on a microplate shaker (600rpm).

9. Aspirate each well and wash as step 6.
10. Add 100 µl of TMB substrate solution into each well. Incubate for 20-30 mins at RT with shaking (600rpm). Avoid exposure to light.
11. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
12. Read the OD with a microplate reader at 450nm (with a reference wavelength between 620nm and 650nm) within 10 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Adrenaline (ng/ml)	0	1	4	15	50	200
Adrenaline (nmol/L)	0	5.5	22	82	273	1092
Conversion	Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/L)					

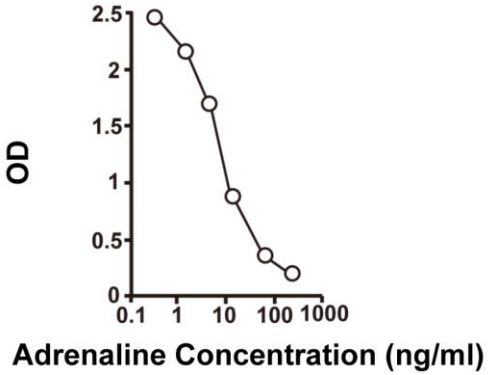
6. The concentration of urine samples and controls can be read directly from the standard curve.

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

7. The read concentrations of plasma samples have to be divided by 30.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

LOD: Urine: 0.9 ng/ml; Plasma: 10 pg/ml

LOQ: Urine: 0.7 ng/ml; Plasma: 18 pg/ml

Assay Range

0.7-200 ng/ml (Urine)

18-6667 pg/ml (Plasma)

Specificity

	Substance	Cross Reactivity (%)
		Adrenaline
Cross Reactivity	Derivatized Adrenaline	100
	Derivatized Noradrenaline	0.13
	Derivatized Dopamine	< 0.01
	Metanephrine	0.18
	Normetanephrine	< 0.01
	3-Methoxytyramine	< 0.01
	3-Methoxy-4-hydrophenylcol	< 0.01
	Tyramine	< 0.01
	Phenylalanine	< 0.01
	Caffeinic acid	< 0.01
	L-Dopa	< 0.01
	Homovanillic acid	< 0.01
	Tyrosine	< 0.01
	3-Methoxy-4-hydroxymandelic acid	< 0.01

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 10.95% and CV value of inter-assay precision was 14.3%.

Recovery

94-120% (urine); 88-117% (plasma)

Linearity

92-123% (urine); 94-115% (plasma)