Adrenaline Research ELISA Kit

Enzyme Immunoassay for the quantification Adrenaline in various biological sample types including EDTA-Plasma, Cell culture supernatants and other biological samples types.

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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 assay employs the competitive enzyme immunoassay technique. An antigen Adrenaline has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Adrenaline present compete for the fixed number of antibody binding site. After washing away any unbound substances, the antibody bound to the solid phase is detected by using TMB as a substrate. The reaction is monitored at 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Microtiter Plate	12 x 8 wells	4°C, ready for use
Extraction Plate (coated with boronate affinity gel)	2 x 48 wells	4°C, ready for use
Adrenaline coated microtiter strips	12 x 8 wells	4°C, ready for use
Standard A-F (0, 0.5, 1.5, 5, 20, 80 ng/ml)	6 x 4 ml	4°C, ready for use
Control 1 (3 ng/ml ± 40%)	4 ml	4°C, ready for use
Control 2 (10 ng/ml ± 40%)	4 ml	4°C, ready for use
Acylation Buffer	20 ml	4°C, ready for use
Acylation Reagent	3 ml	4°C, ready for use
TE Buffer	4 ml	4°C, ready for use
Coenzyme (S-adenosyl-L-methionine)	4 ml	4°C, ready for use

Enzyme (COMT)	4 vial	Lyophilized
Enzyme-conjugated Antibody	12 ml	4°C, ready for use
Adrenaline antiserum	6 ml	4°C, ready for use
Adjustment buffer	4 ml	4°C, ready for use
50X Wash buffer	20 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
HCl	20 ml	Ready for use
STOP solution	12 ml	4°C, ready for use

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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Store at 2-8°C up to 1 month.
- Enzyme solution: Reconstitute the Enzyme (COMT) vial with 1 ml of distilled water. Add 0.3 ml Coenzyme followed by 0.7 ml Adjustment Buffer. The total volume of enzyme solution is 2 ml.
- *Note: The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!*

SAMPLE COLLECTION & STORAGE INFORMATION

Storage: Up to 6 hours at 2 – 8 °C; for longer periods (up to 6 months) at- 20°C or – 80 °C.

Advice for the preservation of the biological sample: to prevent catecholamine degradation add EDTA (final concentration 1 mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

Preparation: The Adrenaline Research ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.

• Avoid excess of acid: excess of acid might exceed the buffer capacity of the extraction buffer. A pH > 7.0 during the extraction is mandatory.

• Prevent catecholamine degradation by adding preservatives to the sample (see above).

• Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of adrenaline. If your samples already contain high amounts of perchloric acid, neutralize the sample prior to the extraction step.

• Tissue samples can be homogenised in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions, adrenaline is positively charged which reduces binding to proteins and optimizes solubility.

• Avoid samples that contain substances with a cis-diol structure. These will reduce the recovery of the adrenaline.

• It is advisable to perform a “Proof of Principle” to determine the recovery of the adrenaline in your samples. Prepare a stock solution of Adrenaline. Add small amounts (to change the native sample matrix as less as possible) of the

stock solutions to the sample matrix and check the recovery.

• The used sample volume determines the sensitivity of the test. Determine the sample volume needed to determine the adrenaline in your sample by testing different amounts of sample volume.

ASSAY PROCEDURE

- **Extraction and acylation**

- If you have sample volumes between 1-100 µl follow 1.1
- If you have sample volumes between 100-500 µl follow 1.2
- If you have sample volumes between 500-750 µl follow 1.3

1.1. Pipette 10 µl standards, 10 µl controls and 1-100 µl samples into the respective wells of the Extraction Plate. Fill up each well with distilled water to a final volume of 100 µl.

1.2. Pipette 10 µl standards, 10 µl controls and 100-500 µl samples into the respective wells of the Extraction Plate. Fill up each well with distilled water to a final volume of 500 µl.

1.3. Pipette 10 µl standards, 10 µl controls and 500-750 µl samples into the respective wells of the Extraction Plate. Fill up each well with distilled water to a final volume of 750 µl.

2. Add 25 µl TE buffer into all wells. Cover the plate with Adhesive Foil. Incubate 1 hour at RT on a microplate shaker (600 rpm).

3. Aspirate each well and wash, repeating the process 1 times for a total 2 washes. Wash by filling each well with 1× Wash Buffer (1 ml) using a squirt bottle, manifold dispenser, or autowasher. Shake the plate at RT for 5 min on a microplate shaker (600 rpm). 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.

4. Add 150 µl Acylation Buffer into all wells.
5. Add 25 µl Acylation Reagent into all wells.
6. Cover wells and shake 20 minutes at RT on a microplate shaker (600 rpm).
7. Wash as according to step 3.
8. Add 100 µl HCl into all wells.
9. Cover plate with Adhesive Foil. Shake the plate at RT (20-25°C) for 10 min on a microplate shaker (approx. 600 rpm).

   Note: Do not decant the supernatant thereafter! 90 µl of the supernatant is needed for the subsequent enzymatic conversion.

- **Enzymatic Conversion**

1. Add 90 µl extracted standards, controls and samples into the respective wells of Microtiter plate.
2. Add 25 µl Enzyme solution into all wells. (please refer to REAGENT PREPARATION section)
3. Cover wells with Adhesive Foil and shake the plate at RT (20-25°C) for 1 min on a microplate shaker (approx. 600 rpm).
4. Incubate for 2 hours at 37°C.
5. Note: 100 µl of the supernatant is needed for the subsequent Adrenaline ELISA procedure.

- **Adrenaline ELISA**

1. Add 100 µl of standards, controls and samples from the Microtiter plate into the respective Adrenaline-coated Microtiter strips.
2. Add 50 µl Adrenaline antiserum into all wells.
3. Cover wells with Adhesive Foil and shake the plate at RT (20-25°C) for 1 min on a microplate shaker (approx. 600 rpm).
4. Incubate for 15-20 hours at 2-8°C.
5. Remove the foil. Aspirate each well and wash, repeating the process 3 times for a total 4 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.
6. Add 100 µl Enzyme-conjugated Antibody into all wells. Cover the wells and incubate at RT for 30 minutes on a microplate shaker (approx. 600 rpm).
7. Wash as according to step 5.
8. Add 100 µl of TMB substrate to each well. Incubate for 20-30 minutes at room temperature on a microplate shaker (approx. 600 rpm) in dark.
9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm immediately. (Optional: read at 620-650 nm as reference wavelength)
    Note: In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

CALCULATION OF RESULTS

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

2. Using semi-logarithmic 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.

$$\text{Correction factor} = \frac{10\ \mu\text{l}\ (volume\ of\ standards\ extracted)}{sample\ volume\ (\mu\text{l}\ )\ extracted}$$

Example:

750 µl of the sample is extracted and the concentration taken from the standard curve is 0.45 ng/ml adrenaline.

Correction factor = 10/750 = 0.013

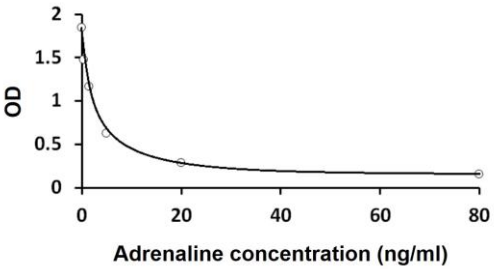
Concentration of the sample = 0.45 ng/ml x 0.013 = 0.006 ng/ml = 6 pg/ml adrenaline

5. Refer to the table at below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Adrenaline (ng/ml)	0	0.5	1.5	5	20	80
Adrenaline (nmol/L)	0	2.73	8.19	27.3	109.2	436.8
Conversion	Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/L)					

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

The minimum detectable dose (MDD) of Adrenaline ranged from 0-80 ng/ml.

The mean MDD was 0.25 ng/ml X correction factor

Analytical Sensitivity (750 µl undiluted sample): 3.3 pg/ml

Functional Sensitivity (750 µl undiluted sample): 5 pg/ml

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 12.3% and inter-assay precision was 13.1%.

Recovery

Human EDTA-Plasma: 89.4-128.3%

Cell culture medium: 81.6-109.6%

Specificity:

	Substance	Cross Reactivity (%)
		Adrenaline
Cross Reactivity	Derivatized Adrenaline	100
	Derivatized Noradrenaline	0.2
	Derivatized Dopamine	< 0.0007
	Metanephrine	0.64
	Normetanephrine	0.0009
	3-Methoxytyramine	< 0.0007
	3-Methoxy-4-hydrophenylcol	0.03
	Tyramine	< 0.0007
	Phenylalanine	< 0.0007
	Caffeinic acid	< 0.0007
	L-Dopa	< 0.0007
	Homovanillic acid	< 0.0007
	Tyrosine	< 0.0007
	3-Methoxy-4-hydroxymandelic acid	< 0.0007