ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1960-20

Norepinephrine/Noradrenaline

High sensitivity ELISA KIT # 1960-20

For Quantitative Determination of Norepinephrine in Plasma & Urine

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Noradrenaline ELISA KIT # 1960-20

ELISA Kit for the quantitative determination of Noradrenaline (norepinephrine) in plasma. For in vitro diagnostic use only. : Kit Contents (96 tests):

Components	Cat. No.
Adhesive Foils, Qty:4	196021F
Wash buffer (50X), 20 ml	196022-WB
Enzyme **Conjugate** (anti-rabbit IgG-HRP) 1x12 ml	196023-EC
Substrate 1x12 ml	196020-SU
Stop Solution, 12 ml	196020-ST
Noradrenaline-**normetanephrine** microwell strips (96 wells, 12 x 8 wells)	196020-24
Noradrenaline Antiserum, 6 ml (Yellow color, yellow cap)	196020-25
Adjustment Buffer, 4 ml	196020-26
Noradrenaline **Stds. A-F** (0, 5, 20, 75, 250, 1000 ng/ml) 6 vials x 4 ml each	196020-27A-F
Noradrenaline **Control 1** (4 ml) Noradrenaline **Control 2** (4 ml)	196020-C1 196020-C2
Acylation Buffer, 20 ml	196020-AB
Acylation Reagent, 3 ml	196020-AR
Assay Buffer, 6 ml	196020-28
Coenzyme, S-adensyl-L-methionine, 4 ml	196020-CE
Enzyme COMT, 2X1 ml (**Lyophilized**)	196020-EZ
Extraction Buffer, 6 ml	196020-EB
Extraction Plate 2X48 wells coated with boronate affinity gel	196020-EP
Diluted Hydrochloric Acid, 20 ml (yellow color)	196020-HCL
Complete Instruction **Manual**	M-1960-20

General Information

Norepinephrine (abbreviated norepi or NE), or noradrenaline (BAN) (abbreviated NA, NAd, or norad), is a catecholamine with multiple roles including as a hormone and a neurotransmitter. Areas of the body that produce or are affected by norepinephrine are described as noradrenergic. One of the most important functions of norepinephrine is its role as the neurotransmitter released from the sympathetic neurons to affect the heart. An increase in norepinephrine from the sympathetic nervous system increases the rate of contractions in the heart. As a stress hormone, norepinephrine affects parts of the brain, such as the amygdala, where attention and responses are controlled. Along with epinephrine, norepinephrine also underlies the fight-or-flight response, directly increasing heart rate, triggering the release of glucose from energy stores, and increasing blood flow to skeletal muscle. It increases the brain's oxygen supply. Norepinephrine can also suppress neuroinflammation when released diffusely in the brain from the locus coeruleus.

Analytical Sensitivity

Noradrenaine: **Urine** 1.5 ng/ml **Plasma** 50 pg/ml

Precision (intra-assay)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
high	135	20.0	15.0
medium	92.7	9.0	9.8
low	24.4	3.9	16.1

Recovery Noradrenaline (Human EDTA-Plasma)

	Mean (%)	Range (%)
Urine	109	83-115
Plasma	97	85-108

Linearity

Range	Range	Serial dilution upto	Range %
Urine	20-339	1:16	85-223
Plasma	318-2436	1:8	84-123

Method Comparison

Noradrenaline HPLC=1.27 ELISA: 0.04 r=0.96; n=30

* The concentrations were assessed using both the ELISA and the HPLC method (external QC samples from UK NEQAS). The correlation between ELISA and HPLC is excellent. This means, that the ELISA measure equally good when compared to the UK NEQAS HPLC data. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.

Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions. It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative. The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

Interference Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

Precautions Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation. All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

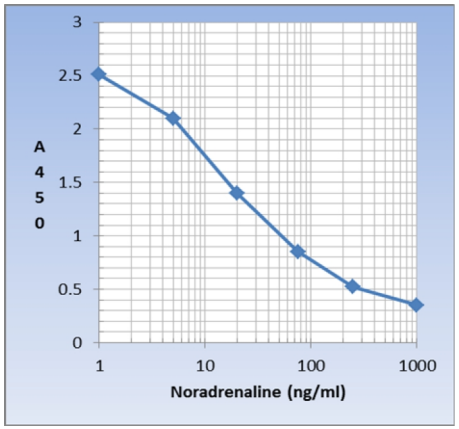
QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC Report.If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.
In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm

Typical calibration curve



Example. Do not use for calculation!

/2-ADI-ELISA_Arif

Expected Reference Values

Noradrenaline: **Plasma** < 600 pg/mL **Urine** <90 ug/day (535 nmol/day)

Analytical Specificity

Substance: **Cross Reactivity%)**
Derivatized Adrenaline: 0.14
Derivatized Noradrenaline: 100
Derivatized Dopamine: 0.2
Metanephrine: < 0.003
Normetanephrine: 0.48
3-Methoxytyramine:< 0.003
3-Methoxy-4-hydroxyphenylglycol: 0.01
Tyramine: < .003, Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid: < .003%

PRINCIPLE OF THE TEST

Noradrenaline (norepinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then derivatized enzymatically. The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analytes compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI's ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

EDTA-**Plasma** should be used. Do not use haemolytic or lipemic samples. Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at - 20°C. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Urine: Spontaneous or 24-hours urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used. Storage: for longer periods (up to 6 months) at -20°C**.** Avoid exposure to direct sun light!

Preparation of the reagent:

Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). store at 4oC.

Enzyme Solution: Reconstitute the content of the vial labeled 'Enzyme' with 1 mL distilled water and mix thoroughly. **Add 0.3 mL** of **Coenzyme** followed by **0.7 mL of Adjustment Buffer**. The total volume of the **Enzyme Solution is 2.0 mL**.

The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10 -15 minutes in advance). Discard after use!

STORAGE AND STABILITY

Store the reagents at 2 - 8 □C until expiration date. Do not use components beyond the expiry date. indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE). .Read the manual very carefully and get familiar with the supplied reagents, preparations and their use in this test.* **Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.**

A. Sample preparation, extraction and acylation

1. Pipette **10 µL** of standards, controls, urine samples and **300 µL** of plasma samples into the respective wells of the Extraction Plate.

2. Add **250 µL** of distilled water to the wells with standards, controls and urine samples.

3. Pipette **50 µL** of Assay Buffer into all wells

4. Pipette **50 µL** of Extraction Buffer into all wells

5. Cover plate with adhesive foil and incubate **30 min** at RT (20-25°C) on a shaker (approx. 600 rpm).

6. Remove the foil. Empty plate and blot dry by tapping the inverted plate on absorbent material.

7. Pipette **1 mL** of Wash Buffer into all wells. Incubate the plate for 5 min at RT (20-25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.

8. Pipette another **1 mL** of Wash Buffer into all wells. Incubate the plate for 5 min at RT (20-25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.

9. Pipette **150 µL** of Acylation Buffer into all wells.
10. Pipette **25 µL** of Acylation Reagent into all wells.
11. Incubate **15 min** at RT (20-25°C) on a shaker (approx. 600 rpm).
12. Empty plate and blot dry by tapping the inverted plate on absorbent material.
13. Pipette **1 mL** of Wash Buffer into all wells. Incubate the plate for **10 min** at RT (20-25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
14. Pipette **150 µL** of Hydrochloric Acid into all wells.
15. Cover plate with adhesive foil. Incubate **10 min** at RT (20-25°C) on a shaker (approx. 600 rpm). Remove the foil and discard.

Do not decant the supernatant thereafter!
The following volumes of the supernatant are needed for the subsequent ELISA:
Noradrenaline 20 µL

B. Noradrenaline ELISA

1. Pipette **25 µl** of the Enzyme Solution (refer to page-2) into all wells of the Noradrenaline Microtiter Strips.
2. Pipette **20 µL** of the extracted standards, controls and samples into the appropriate wells.
3. Incubate for **30 min** at RT (20-25°C) on a shaker (approx. 600 rpm).
4. Pipette **50 µL** of the Noradrenaline Antiserum into all wells and cover plate with Adhesive Foil.
5. Incubate for **2 hours** at RT (20-25°C) on a shaker (approx. 600 rpm).
6. Remove the foil. Discard or aspirate the content of the wells and wash each well **3 times** thoroughly with 300 µL Wash Buffer. Blot dry by tapping the inverted plate on absorbent material.
7. Pipette **100 µL** of the Enzyme Conjugate into all wells.
8. Incubate for **30 min** at RT (20-25°C) on a shaker (approx. 600 rpm).
9. Discard or aspirate the content of the wells and wash each well **3 times** thoroughly with 300 µL Wash Buffer. Blot dry by tapping the inverted plate on absorbent material.
10. Pipette **100 µL** of the Substrate into all wells and incubate for 25 ±5 min at RT (20-25°C) on a shaker (approx. 600 rpm). Avoid exposure to direct sun light!
11. Add **100 µL** of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
12. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

Calculation of results:

The calibration curves are obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use a non-linear regression for curve fitting (e.g. spline, 4-parameter, akima).

	Concentration of the standards					
	A	B	C	D	E	F
Noradrenaline (ng/ml)	0	5	20	75	250	1000
Noradrenaline (nmol/L)	0	30	118	443	1478	5910
Conversion	Noradrenaline (ng/mL)x5.91= (nmol/L)					

Urine samples and controls:

The concentrations of the urine samples and the Controls 1 & 2 can be read directly from the standard curve.
Calculate the 24 h excretion for each urine sample: **µg/24h = µg/L x L/24h**

Plasma samples:

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