Noradrenaline ELISA Kit

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

Catalog number: ARG80475

Package: 96 wells

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PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. Noradrenaline are first extracted by using a cis-diol-specific affinity gel, acylated and derivatized enzymatically. An antigen Noradrenaline has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Noradrenaline 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	1 x 96 wells	4°C, ready for use
Extraction Plate (coated with boronate affinity gel)	2 x 48 wells	4°C, ready for use
Noradrenaline-coated microtiter strips	12 x 8 wells	4°C, ready for use
Standard A-F (0, 0.2, 0.6, 2, 8, 32 ng/ml)	6 x 4 ml	4°C, ready for use
Control 1 (1.2 ng/ml ± 40%)	4 ml	4°C, ready for use
Control 2 (4 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 vials	Lyophilized
Enzyme-conjugated Antibody	12 ml	4°C, ready for use
Noradrenaline 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	4°C, Ready for use
STOP solution	12 ml	4°C, ready for use
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as reference wavelength)
- Orbital microplate shaker: 3 mm (0.1118 in) 600 ± 10 rpm or 19 mm (0.75 in) 170 ± 10 rpm.
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the unopened reagents at 2- 8°C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.
- All kit reagents and specimens should be brought to room temperature (20 – 25 °C) 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.
- In rare cases residues of the blocking and stabilizing reagent can be seen in the wells of Noradrenaline-coated microtiter strips as small, white dots or lines. These residues do not influence the quality of the product.

- 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.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. E.g. dilute the 20 ml of 50X Wash Buffer into distilled water to a final volume of 1000 ml. The diluted wash buffer can be stored at 2 – 8 °C for 1 month.
- Enzyme solution: Reconstitute the lyophilized "Enzyme (COMT)" with 1ml of distilled water and mix well. Add 0.3 ml of Coenzyme followed by 0.7 ml of Adjustment buffer. The total volume of Enzyme solution is 2ml. 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 Noradrenaline 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 noradrenaline degradation by adding preservatives to the sample (see above).

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

• Tissue samples can be homogenized in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions, Noradrenaline 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 Noradrenaline.

• It is advisable to perform a “Proof of Principle” to determine the recovery of the Noradrenaline in your samples. Prepare a stock solution of Noradrenaline. 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 Noradrenaline 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

Note: Within a run it is only possible to measure samples with the same volume!

1.1. Pipette **10 µl** of **standards, 10 µl** of **controls** and **1-100 µl** of **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** of **standards, 10 µl** of **controls** and **100-500 µl** of **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** of **standards, 10 µl** of **controls** and **500-750 µl** of **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** of **TE buffer** into all wells. Cover the plate with Adhesive Foil. Incubate **1 hour at RT (20- 25°C)** 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** of **Acylation Buffer** into all wells.
5. Add **25 µl** of **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** of **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** of **extracted standards, controls and samples** into the respective wells of **Microtiter plate**.
2. Add **25 µl** of **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) to mix thoroughly.
4. Incubate for **2 hours at 37°C.**

   Note: **100 µl** of the supernatant is needed for the subsequent Noradrenaline ELISA procedure.

- **Noradrenaline ELISA**

1. Add **100 µl** of **standards, controls and samples** from the Microtiter plate (of Enzymatic Conversion process) into the respective **Noradrenaline-coated Microtiter strips**.
2. Add **50 µl** of **Noradrenaline 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** of **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 substrat**e 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) It is recommended read the absorbance within 10 minutes after adding the stop solution.

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.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor.

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

Example

750 µl of the sample is extracted and the concentration taken from the standard curve is 0.15 ng/ml noradrenaline.

Correction factor = 10/750 = 0.013

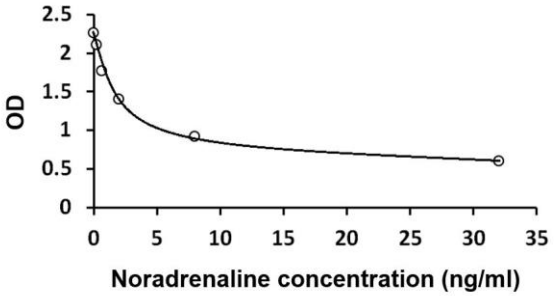
Concentration of the sample = 0.15 ng/ml x 0.013 = 0.002 ng/ml = 2 pg/ml noradrenaline

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

	Concentration of standards					
Standard	A	B	C	D	E	F
Noradrenaline (ng/ml)	0	0.2	0.6	2	8	32
Noradrenaline (nmol/L)	0	1.2	3.5	12	47	189
Conversion	Noradrenaline (ng/ml) x 5.91 = Noradrenaline (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 Noradrenaline ranged from 0-32 ng/ml. The mean MDD was 0.1 ng/ml X correction factor

Analytical Sensitivity (750 µl undiluted sample): 1.3 pg/ml

Functional Sensitivity (750 µl undiluted sample): 2 pg/ml

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 11.9% and inter-assay precision was 10.77%.

Recovery

Human EDTA-Plasma: 104.8-125.6%

Cell culture medium: 70.6-124.7%

Cross Reactivity

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