



Bi-CAT ELISA

For quantitative determination of Epinephrine and Norepinephrine in plasma and urine

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	17-BCTHU-E02
Size:	2 x 96 wells
Version:	23-Mar-2010 - ALPCO 4/19/2010

ALPCO Diagnostics

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1. Principle of the test

This kit is for the quantitative determination of epinephrine and norepinephrine in plasma and urine. They are extracted using a cis-diol-specific affinity gel, acylated, and then derivatized enzymatically. The competitive ELISA kit uses the microplate format. The antigen is bound to the solid phase of the microplate. The derivatized standards, controls, and samples and 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. 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.

2. Storage and stability

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

3. Contents of the kit

BA 1611	ACYL-BUFF	Acylation Buffer	1 x 20 mL	ready for use
BA 1612	ACYL-REAG	Acylation Reagent	2 x 1.5 mL	ready for use
BA 1613	ASSAY-BUFF	Assay Buffer	2 x 4 mL	ready for use, contains 1 M HCl
BA 1614	COENZYME	Coenzyme	2 x 0.75 mL	ready for use, S-adenosyl-L-methionine
BA 1615	ENZYME	Enzyme	4 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
BA 1617	EXTRACT-BUFF	Extraction Buffer	2 x 4 mL	ready for use
BA 1618	EXTRACT-PLATE 48	Extraction Plate	2 x 48 wells	coated with boronate affinity gel
BA 1619	HCL	Hydrochloric Acid	1 x 20 mL	ready for use, yellow colored, contains 0.025 M HCl
BA 3050	ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
BA 10-0025	WASH-CONC 25x	Washbuffer Concentrate	3 x 20 mL	concentrate, dilute content with dist. water to a final volume of 500 mL
BA 10-0040	CONJUGATE	Enzyme Conjugate	2 x 11 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA 10-0055	SUBSTRATE	Substrate	2 x 11 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA 10-0080	STOP-SOLN	Stop Solution	2 x 11 mL	ready for use, containing 0.25 M H ₂ SO ₄
BA 10-0090	FOILS	Adhesive Foil	2 x 4	ready for use
BA 10-0110	EPI-AS	Epinephrine Antiserum	1 x 6 mL	from rabbit, ready for use, blue colored, blue screw cap
BA 10-0131	EPI MN	Epinephrine-Metanephrine Microstrips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue colored
BA 10-0210	NOR-AS	Norepinephrine Antiserum	1 x 6 mL	from rabbit, ready for use, yellow colored, yellow screw cap
BA 10-0231	NOR NMN	Norepinephrine-Normetanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow colored
BA 10-1601	STANDARD A	Standard A	1 x 2 mL	ready for use
BA 10-1602	STANDARD B	Standard B	1 x 2 mL	ready for use
BA 10-1603	STANDARD C	Standard C	1 x 2 mL	ready for use
BA 10-1604	STANDARD D	Standard D	1 x 2 mL	ready for use
BA 10-1605	STANDARD E	Standard E	1 x 2 mL	ready for use
BA 10-1606	STANDARD F	Standard F	1 x 2 mL	ready for use
BA 10-1651	CONTROL 1	Control 1	1 x 2 mL	ready for use
BA 10-1652	CONTROL 2	Control 2	1 x 2 mL	ready for use

4. Additional materials and equipment required but not provided in the kit

- Calibrated variable precision micropipettes (e.g., 10-100 µL / 100-1,000 µL)
- Microplate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towels), distilled or deionized water, vortex mixer

5. Sample collection and storage

Plasma

EDTA-Plasma. Do not use hemolytic and lipemic samples.

Storage: up to 6 hours at 2 - 8°C, for longer periods (up to 6 months) at - 20°C.

Repeated freezing and thawing should be avoided.

Urine

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl.

Storage: for longer periods (up to 6 months) at -20°C.

Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicates are recommended.

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 500 mL. Store the diluted Wash Buffer Concentrate (Wash Buffer) at 2 – 8°C. Shelf life: please refer to the expiry date indicated on the kit.

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 immediately prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!

6.2 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.



Do not decant the supernatant thereafter!

The following volumes of the supernatant are needed for the subsequent ELISA:

Epinephrine	100 µL
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Norepinephrine	20 µL
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6.3 Epinephrine ELISA

1.	Pipette 25 µl of the Enzyme Solution (refer to 6.1) into all wells of the Epinephrine Microstrips .
2.	Pipette 100 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 Epinephrine 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 20-30 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Avoid exposure to direct sunlight!</i>
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.

6.4 Norepinephrine ELISA

1.	Pipette 25 µl of the Enzyme Solution (refer to 6.1) into all wells of the Norepinephrine Microstrips .
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 Norepinephrine 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 contents 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 20-30 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Avoid exposure to direct sunlight!</i>
11.	Add 100 µL of the Stop Solution to each well and shake the microplate 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.

7. Calculation of results

Standard	Concentration of the standards					
	A	B	C	D	E	F
Epinephrine (ng/mL)	0	1	4	16	64	256
Epinephrine (nmol/L)	0	5.46	21.8	87.4	349	1,398
Norepinephrine (ng/mL)	0	4	16	64	256	1,024
Norepinephrine (nmol/L)	0	23.6	94.6	378	1,513	6,052
Conversion:	Epinephrine (ng/mL) x 5.46 = Epinephrine (nmol/L) Norepinephrine (ng/mL) x 5.91 = Norepinephrine (nmol/L)					

The calibration curves are obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis).

Use a non-linear regression for curve fitting (e.g., spline, 4-parameter, akima).

Urine samples and controls:

The concentrations of the **urine samples** and the **Controls 1 & 2** can be read directly from the standard curve.

Plasma samples:


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

7.1 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.

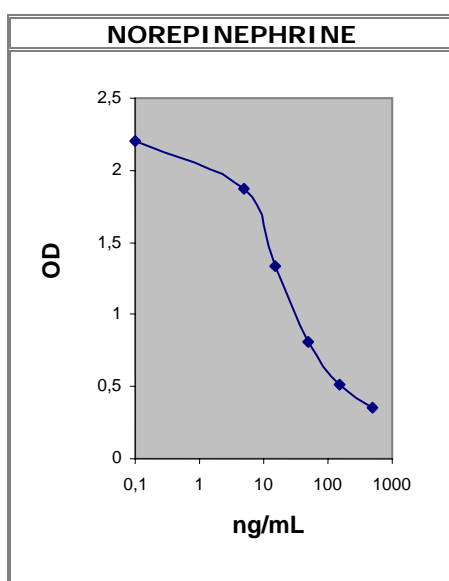
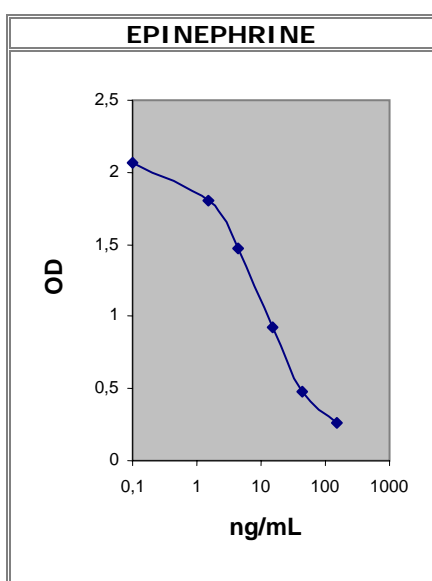
7.2 Calibration

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. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

 *In cases of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.*

7.3 Typical calibration curves

 *Examples. Do not use for calculation!*



8. Assay characteristics

Expected values	Epinephrine		Norepinephrine	
	Urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)	
	Plasma	< 100 pg/mL	< 600 pg/mL	

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD		
		Epinephrine	Norepinephrine
	Urine	0.33 ng/mL	1.33 ng/mL
	Plasma	11 pg/mL	44 pg/mL

Analytical Specificity (Cross-reactivity)	Substance	Cross-reactivity (%)	
		Norepinephrine	Epinephrine
	Derivatized Epinephrine	0.14	100
	Derivatized Norepinephrine	100	0.20
	Derivatized Dopamine	0.2	< 0.0007
	Metanephrine	< 0.003	0.64
	Normetanephrine	0.48	0.0009
	3-Methoxytyramine	< 0.003	< 0.0007
	3-Methoxy-4-hydroxyphenylglycol	0.01	0.03
	Tyramine	< 0.003	< 0.0007
Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007	

Precision							
Intra-assay				Inter-assay			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Norepinephrine	1	24.4 ± 3.9	16.1	Norepinephrine	1	39.8 ± 3.4	8.5
	2	92.7 ± 9.0	9.8		2	135 ± 20	15.0
Epinephrine	1	2.5 ± 0.4	15.0	Epinephrine	1	8.8 ± 1.1	13.2
	2	11.7 ± 0.8	6.9		2	34.2 ± 5.2	15.4

Linearity			Range	Serial dilution up to	Range (%)
	Norepinephrine	Urine	20 - 339 ng/mL	1:16	85 - 123
		Plasma	318 - 2,436 pg/mL	1:8	84 - 123
	Epinephrine	Urine	4.6 - 81.4 ng/mL	1:16	86 - 124
Plasma		92 - 545 pg/mL	1:8	81 - 121	

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Norepinephrine	Urine	109	83 - 115	
		Plasma	97	85 - 108	
	Epinephrine	Urine	107	84 - 119	
Plasma		92	80 - 113		

Method Comparison versus HPLC*	Norepinephrine	HPLC = 1.27 ELISA - 0.04	r = 0.96; n = 30
	Epinephrine	HPLC = 1.17 ELISA - 0.06	r = 0.99; 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 measures equally good when compared to the UK NEQAS HPLC data. Please keep in mind that the UK control values are the mean of about 40 different HPLC users, and always contain one pathological sample per group.

9. Advice on handling the test

9.1 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, RILIBAK, 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 normal range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact ALPCO for further instructions.

9.2 Complaints

In case of complaints, please submit a written report containing all data as to how the test was conducted, the results received, and a copy of the original test printout to ALPCO.

9.3 General Information

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results.

9.4 Disposal

Residual substances and/or all remaining chemicals, reagents, and ready for use solutions, are special refuse. Their disposal is subject to the laws and regulations of the federation and the countries. Inform the responsible authorities or refuse disposal enterprises about the removal of special refuse. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic and waste law.

The appropriate material safety data sheets of the individual products are available upon request. The material safety data sheets correspond to the standard: ISO 11014-1.

9.5 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 affect the results. Use no kit components beyond the expiry date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

9.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and samples with skin. No smoking, eating, or drinking in the laboratory where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hands thoroughly before exiting the laboratory. 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 plumbing and may form highly explosive metal azides. When clearing up, rinse thoroughly with large volumes of water to prevent such formation. All reagents of this test kit which contain human or animal serum or plasma have been tested and confirmed negative for HIV 1/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.