DRG® CatCombi ELISA (EIA-3175)

CE IVD

REVISED 31 JULY 2009 (VERS. 4.0)

RUO IN THE USA

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* separate quantitative determination of Adrenaline (epinephrine) and Noradrenaline (norepinephrine) in human plasma and urine. In the United States, this kit is intended for Research Use Only.

SUMMARY AND EXPLANATION

The catecholamines Adrenaline, Noradrenaline and dopamine are synthesized in the adrenal medulla, the sympathetic nervous system and in the brain. They influence virtually all tissues and are involved together with other hormonal and neuronal systems in the regulation of a wide variety of physiological processes.

As catecholamines and their metabolites metanephrine and normetanephrine are secreted in increasing amounts in a number of diseases, they may be used for diagnostic purposes.

In this context, diagnosis and the follow-up of tumor diseases of the nervous system are of special importance. This applies primarily to the pheochromocytoma, but also the neuroblastoma and the ganglioneuroma.

Because of the extraction step at the beginning of the assay, the customer is able to use all kinds of animal species material. It works for rats, mice and others. The chemical structure of the catecholamines is identical in all animals.

TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with a goat anti rabbit antibody. The added liquid antibody, directed towards an epitope of an antigen molecule binds to the plate within the incubation time. The antigen of the sample is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After the substrate reaction the intensity of the developed color is proportional to the amount of the antigen. Results of samples can be determined directly using the standard curve.

WARNINGS AND PRECAUTIONS

1. For in-vitro diagnostic use only. For professional use only. In the United States, this kit is intended for Research Use Only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Observe lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available upon request.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

SPECIMEN COLLECTION AND STORAGE

⚠	The in-vivo catecholamine and metanephrines release is influenced by several foods and drugs. Vitamin B, coffee and bananas, alpha-methyldopa, MAO and COMT inhibitors as well as medications related to hypertension should be discontinued for at least 72 h prior to specimen collection.

Plasma (EDTA)

⚠	The blood sample should be stored at 2-8°C until centrifuged to separate the plasma within 2 h after blood collection.

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light.
Stability:	6 h	1 mon	Avoid repeated freeze-thaw cycles. Ship samples frozen.

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle containing 10 - 15 mL of 6 N HCl as preservative. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

Storage:	≤ -20°C (Aliquots)	Keep away from heat or direct sun light.
Stability:	6 mon	Avoid repeated freeze-thaw cycles.

CE IVD

REVISED 31 JULY 2009 (VERS. 4.0)

RUO IN THE USA

MATERIALS SUPPLIED

⚠	The reagents provided with this kit are sufficient for up to 48 single determinations in the extraction procedure (6 standards, 2 controls, 40 patient samples) and up to 48 duplicates in the ELISA for each Adrenaline and Noradrenaline in plasma and urine. Additional reagents are available upon request.

⚠	The microtiter plate can be used for Adrenaline and Noradrenaline.

Quantity	Symbol	Component
2 x 12x8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 6 x 2.5 mL	CAL A-F	**Standard A-F** Adrenaline: 0; 1.5; 5.0; 15; 50; 150 ng/mL (0; 8; 27; 82; 273; 819 nmol/L) Noradrenaline: 0; 5.0; 15; 50; 150; 500 ng/mL (0; 30; 89; 296; 887; 2955 nmol/L) Dopamine: 0; 60; 180; 585; 2300; 11470 ng/mL (0; 392; 1175; 3819; 15014; 74876 nmol/L) Ready to use. Contains:: [-] Adrenaline, [-] Noradrenaline [-] Dopamine (biologically active), 0.1 M HCl.
1 x 2 x 2.5 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: [-] Adrenaline, [-] Noradrenaline, [-] Dopamine (biologically active), 0.1 M HCl. Concentrations / acceptable ranges see vial labels.
2 x 250 µL	ENZCONJ CONC	**Enzyme Conjugate** Concentrate (100x) Contains: antibodies, conjugated to alkaline phosphatase, Tris buffer, HCl, 0.01 % NaN_3.
2 x	EXTRPLATE	**Extraction Plate** (Macrotiter Plate) 24 wells each. Coated with boronate affinity gel.
1 x 60 mL	EXTRBUF	**Extraction Buffer** Pink colored. Ready to use. Contains: 0.016 % NaN_3.
4 x 1.25 mL	COMT LYO	**COMT** lyophilized Contains: Catechol-O-methyltransferase (porcine liver), NaN_3.
1 x 2 mL	COMT ADD	**COMT Additive** Contains: human plasma, stabilizers, 0.01 % Thimerosal.

CE IVD

REVISED 31 JULY 2009 (VERS. 4.0)

RUO IN THE USA

Quantity	Symbol	Component
1 x 7.0 mL	ANTISERUM AD	**Adrenaline Antiserum** Green colored. Ready to use. Contains: antibodies against Adrenaline (rabbit), Buffer, stabilizers.
1 x 7.0 mL	ANTISERUM NAD	**Noradrenaline Antiserum** Blue colored. Ready to use. Contains: antibodies against Noradrenaline (rabbit), Buffer, stabilizers.
4 x 1.25 mL	COENZ	**Coenzyme Solution** Ready to use. Contains: S-Adenosyl-L-Methionine, stabilizers.
2 x 3 mL	ENZBUF	**Enzyme Buffer** Ready to use. Contains: Tris buffer, HCl, stabilizers.
2 x 13 mL	RELEASEBUF	**Release Buffer** Yellow colored. Ready to use. Contains: 0.1 M HCl, indicator.
1 x 3 mL	ACYLREAG	**Acylation Reagent** Ready to use. Contains: dimethylformamide, Ethanol. Caution! Toxic. Highly flammable.
3 x 50 mL	WASHBUF CONC	**Wash Buffer** Concentrate (10x) Contains: Tris buffer, HCl, Tween, 0.2 % NaN_3.
2 x 9 x	PNPP SUBS	**PNPP Substrate Tablets** In one foil packet. Contains: p-nitrophenyl phosphate (PNPP).
2 x 27 mL	PNPP BUF	**PNPP Substrate Buffer** Ready to use. Contains: diethanolamine, water, 0.05 % NaN3.
2 x 15 mL	PNPP STOP	**PNPP Stop Solution** Ready to use. Contains: 1 M NaOH, 0.25 M EDTA.
6 x	FOIL	**Adhesive Foil**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 10; 10-100; 100-1000 µL
2. Orbital shaker (200-900 rpm) (e.g. EAS 2/4, SLT)
3. Vortex mixer
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. Disposable tubes for sample dilution
10. 0.1 N HCL for sample dilution (urine)

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout. A pipetting scheme covering both sample pretreatment and assay is available upon request.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled evenly with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

MANUAL PROCEDURE

PRE-TEST SETUP INSTRUCTIONS

⚠	The contents of the kit for 96 determinations can be divided into 2 separate runs. The volumes stated below are for one run with 2 x 6 strips (96 determinations). **Visible amounts of gel can be separated from surface of extraction plate during extraction. This has no influence on test results.**

Dilution of Samples

Samples suspected to contain concentrations above the highest standard have to be diluted as follows:

Sample	**to be diluted**	**with**	**Remarks**
Plasma	> highest standard	bidist. water	prior to extraction step

Urine	> highest standard	0.1 N HCl	prior to extraction step

Extraction of Samples, Standards and Controls (Extraction Plate) (manual version)

1. Pipette **20 µL** of each **Standard, Control and urine sample** and **500 µL** of each **plasma sample** into the respective wells of the extraction plate. Add **500 µL** of **bidist. water** to all wells except for the **plasma samples** to correct differences of volumes.
2. Pipette **1000 µL** of **Extraction Buffer** into each well.
3. Cover plate with adhesive foil. **Extract 30 min** at **RT (18-25°C)** on an orbital shaker (600–900 rpm).
   During extraction the surface of the liquid should wet the adhesive foil, but the liquid level should not exceed 2/3 of the well. Splashing does not affect results.
4. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel.
5. Pipette **2 mL** of **bidist water** into each well.
6. Cover plate with new adhesive foil.
   Shake 5 min at **RT (18-25°C)** on an orbital shaker (600–900 rpm). Splashing does not affect results.
7. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
8. Pipette **150 µL** of **Extraction Buffer** into each well. To each well add **50 µL** of **Acylation Reagent**. Mix immediately after pipetting.
9. **Extract 20 min** at **RT (18-25°C)** (without adhesive foil) on an orbital shaker (400–600 rpm).
10. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
11. Pipette **2 mL** of **bidist. water** into each well.
12. Cover plate with new adhesive foil. **Shake 5 min** at **RT (18-25°C)** on an orbital shaker (600–900 rpm). Splashing does not affect results.
13. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
14. Pipette **300 µL** of **Release Buffer** into each well.
15. **Shake 30 min** at **RT (18-25°C)** (without adhesive foil) on an orbital shaker (400–600 rpm).

Prepared samples should be assayed the same day. If this is not possible, you can store the extraction plate covered with adhesive foil at 2-8°C over night.

PREPARATION OF LYOPHILIZED OR CONCENTRATED COMPONENTS

Dilute/ dissolve	Component	with	Diluent	Rela-tion	Remarks	Storage	Stability
25 mL	**Wash Buffer**	225 mL	bidist. water	1:10	Mix vigorously.	2-8°C	4 w
110 µL	**Enzyme Conjugate**	11 mL	Wash Buffer (diluted)	1:101	Prepare freshly and use only once. Mix without foaming.	18-25°C	5 h
9	**PNPP Substrate Tablets**	24 mL	PNPP Substrate Buffer		Prepare freshly and use only once.	18-25°C	5 h

TEST PROCEDURE (manual version)

Preparation of COMT Enzyme Solution

⚠ **The COMT Enzyme Solution should be freshly prepared directly before use.**

Dissolve each kit component of lyophilized **COMT** in **1.25 mL bidist. water** and mix the dissolved COMT.*

Then **pipette 1.25 mL** of **Coenzyme Solution** followed by **1.25 mL** of **Enzyme Buffer** and **0.40 mL COMT Additive** to the mixed COMT vials to give a final volume of 4.15 mL of COMT Enzyme Solution per vial.

Pool two (2) vials for 48 determinations of Adrenaline and 48 determinations of Noradrenaline. Solution may be turbid. Mix without foaming. The COMT solution is stable at room temperature for 1 hour.

* If only an aliquot of the COMT solution is needed, the rest of the COMT solution should be frozen immediately in aliquots at -20° C. The COMT solution is stable under these conditions for 1-2 mon.

Enzymatic Derivatization of Samples, Standards and Controls (Microtiter Plate)

⚠	It is recommended to start with Adrenaline. If pipetting with *positive displacement*, give the residual fluid from the tip of the pipette back to the corresponding wells of the extraction plate, otherwise the extracts may not be sufficient for Noradrenaline determination. It is useful to hold the extraction plate in a sloping position. Before use of the Microtiter plates, define and label the wells for Adrenaline and Noradrenaline.

Adrenaline for urine and plasma

1. Pipette **75 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake briefly.
2. Pipette **100 µL** of each extracted **Standard, Control and sample** into the respective wells. During this step hold the pipette tips directly into the COMT solution. Color changes to pink. Shake briefly.
3. Pipette **50 µL** of **Adrenaline Antiserum** (green colored) into each well.
4. Cover plate with adhesive foil. **Incubate 120 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm).

Noradrenaline for urine and plasma

1. Pipette **25 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake briefly.
2. Pipette **25 µL** of each extracted **Standard, Control and sample** into the respective wells. During this step hold the pipette tips directly into the COMT solution. Color changes to pink. Shake briefly.
3. Pipette **50 µL** of **Noradrenaline Antiserum** (blue colored) into each well.
4. Cover plate with adhesive foil. **Incubate 120 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm).

ELISA

The following procedure must be performed for Adrenaline and Noradrenaline.

1. Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 - 300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
2. Pipette **100 µL** of freshly prepared **Enzyme Conjugate** into each well.
3. Cover plate with new adhesive foil. **Incubate 60 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm).
4. Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 - 300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6. Pipette **200 µL** of **Substrate Solution** into each well.
7. **Incubate 40 min** at **RT (18-25°C)** (without adhesive foil) on an orbital shaker (400–600 rpm).
8. Stop the substrate reaction by adding **50 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9. **Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 620-650 nm) within **60 min** after pipetting of the Stop Solution. No air bubbles should be visible.

AUTOMATED PROCEDURE

PRE-TEST SETUP INSTRUCTIONS (automated version)

⚠	The contents of the kit for 96 determinations can be divided into 2 separate runs. The volumes stated below are for one run with 2 x 6 strips (96 determinations). **Visible amounts of gel can be separated from surface of extraction plate during extraction. This has no influence on test results.**

Dilution of Samples

Samples suspected to contain concentrations above the highest standard have to be diluted as follows:

Sample	to be diluted	with	Remarks
Plasma	> highest standard	bidist. water	prior to extraction step
Urine	> highest standard	0.1 N HCl	prior to extraction step

Extraction of Samples, Standards and Controls (Extraction Plate) (automated version)

1. Pipette **30 µL** of each **Standard, Control and urine sample** and **750 µL** of each **plasma sample** into the respective wells of the extraction plate. Add **750 µL** of **bidist. water** to all wells except for the **plasma samples** to correct differences of volumes.
2. Pipette **1000 µL** of **Extraction Buffer** into each well.
3. Cover plate with adhesive foil. **Extract 30 min** at **RT (18-25°C)** on an orbital shaker (600–900 rpm).
   During extraction the surface of the liquid should wet the adhesive foil, but the liquid level should not exceed 2/3 of the well. Splashing does not affect results.
4. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel.
5. Pipette **2 mL** of **bidist water** into each well.
6. Cover plate with new adhesive foil.
   Shake 5 min at **RT (18-25°C)** on an orbital shaker (600–900 rpm). Splashing does not affect results.
7. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
8. Pipette **150 µL** of **Extraction Buffer** into each well. To each well add **50 µL** of **Acylation Reagent**. Mix immediately after pipetting.
9. **Extract 20 min** at **RT (18-25°C)** (without adhesive foil) on an orbital shaker (400–600 rpm).
10. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
11. Pipette **2 mL** of **bidist. water** into each well.
12. Cover plate with new adhesive foil. **Shake 5 min** at **RT (18-25°C)** on an orbital shaker (600–900 rpm). Splashing does not affect results.
13. Remove adhesive foil. Immediately empty plate and eliminate residual fluid on a paper towel. Remove fluid completely.
14. Pipette **450 µL** of **Release Buffer** into each well.
15. **Shake 30 min** at **RT (18-25°C)** (without adhesive foil) on an orbital shaker (400–600 rpm).

Prepared samples should be assayed the same day. If this is not possible, you can store the extraction plate covered with adhesive foil at 2-8°C over night.

Preparation of lyophilized or concentrated components automated version

Dilute/ dissolve	Component	with	Diluent	Rela-tion	Remarks	Storage	Stability
50 mL	**Wash Buffer**	450 mL	bidist. water	1:10	Mix vigorously.	2-8°C	4 w
150 µL	**Enzyme Conjugate**	15 mL	Wash Buffer (diluted)	1:101	Prepare freshly and use only once. Mix without foaming.	18-25°C	5 h
9	**PNPP Substrate Tablets**	24 mL	PNPP Substrate Buffer		Prepare freshly and use only once.	18-25°C	5 h

TEST PROCEDURE (automated version)

Preparation of COMT Enzyme Solution

⚠ **The COMT Enzyme Solution should be freshly prepared directly before use.**

Dissolve each kit component of lyophilized **COMT** in **1.25 mL bidist. water** and mix the dissolved COMT.*

Then **pipette 1.25 mL** of **Coenzyme Solution** followed by **1.25 mL** of **Enzyme Buffer** and **0.40 mL COMT Additive** to the mixed COMT vials to give a final volume of 4.15 mL of COMT Enzyme Solution per vial.

Pool two (2) vials for 48 determinations of Adrenaline and 48 determinations of Noradrenaline. Solution may be turbid. Mix without foaming. The COMT solution is stable at room temperature for 1 hour.

* If only an aliquot of the COMT solution is needed, the rest of the COMT solution should be frozen immediately in aliquots at -20° C. The COMT solution is stable under these conditions for 1-2 mon.

Enzymatic Derivatization of Samples, Standards and Controls (Microtiter Plate)

Adrenaline for urine and plasma

1. Pipette **75 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake plate 1 min.
2. Pipette **100 µL** of each extracted **Standard, Control and sample** into the respective wells. Shake plate 1 min.
3. Pipette **50 µL** of **Adrenaline Antiserum** (green colored) into each well.
4. Cover plate. **Incubate 120 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm).

Noradrenaline for urine and plasma

1. Pipette **25 µL** of freshly prepared **COMT Enzyme Solution** into each well of the **Microtiter Plate**. Shake plate 1 min.
2. Pipette **25 µL** of each extracted **Standard, Control and sample** into the respective wells. Shake plate 1 min.
3. Pipette **50 µL** of **Noradrenaline Antiserum** (blue colored) into each well.
4. Cover plate. **Incubate 120 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm).

ELISA (automated version)

The following procedure must be performed for Adrenaline and Noradrenaline.

1. Discard incubation solution. Wash plate **6 x** with **250 - 300 µL** of diluted **Wash Buffer**.
2. Pipette **100 µL** of **Enzyme Conjugate** into each well.
3. Cover plate. **Incubate 60 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm).
4. Discard incubation solution. Wash plate **6 x** with **250 - 300 µL** of diluted **Wash Buffer**.
5. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution.
6. Pipette **200 µL** of **Substrate Solution** into each well.
7. **Incubate 40 min** at **RT (18-25°C)** on an orbital shaker (400–600 rpm). ***If temperature in automat exceeds 25°C, shorten incubation time to 30 min to avoid signal overflow.***
8. Stop the substrate reaction by adding **50 µL** of **PNPP Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9. **Measure** optical density with a photometer at **405 nm** (Reference-wavelength: 620-650 nm) within **60 min** after pipetting of the Stop Solution.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards.

The concentration of the kit Controls and of the urine samples can be read directly from the corresponding standard curve. Due to the pipetting volume of 500 µL (automated version: 750 µL) for plasma in comparison to 20 µL (automated version: 30 µL) for the standards, the results for plasma samples have to be divided by 25. For units in pg/mL please multiply by 1000.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

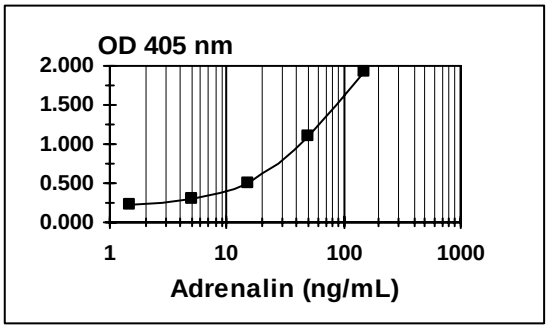
Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

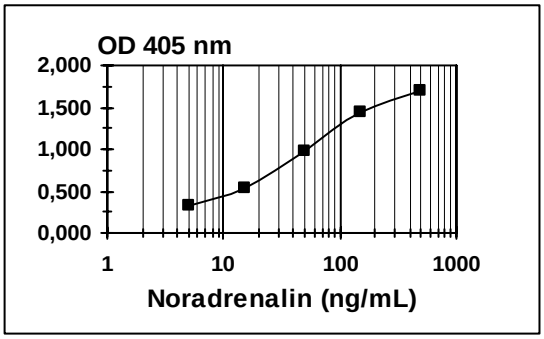
Calculate the 24 h excretion for each urine sample: µg/24h = µg/L x L/24h

Conversion:
Adrenaline (µg/L) x 5.458 = nmol/L
Noradrenaline (µg/L) x 5.911 = nmol/L

Typical Calibration Curve
(Example. Do not use for calculation!)

Standard	Adrenalinee (ng/mL)	Mean OD	OD/OD_{max} (%)
A	0.0	0.183	0.0
B	1.5	0.215	1.8
C	5.0	0.291	6.2
D	15	0.490	17.7
E	50	1.104	53.2
F	150	1.914	100
Standard	Noradrenaline (ng/mL)	Mean OD	OD/OD_{max} (%)
A	0.0	0.223	0.0
B	5.0	0.322	6.7
C	15	0.539	21.3
D	50	0.984	51.2
E	150	1.438	81.8
F	500	1.708	100





EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values: (5 % - 95 % percentile)

It is recommended that each laboratory establishes its own range of normal values.

	Urine		Plasma	
	µg/d	nmol/d	pg/mL	nmol/L
Adrenaline	< 20	< 110	< 125	< 0.68
Noradrenaline	< 90	< 535	< 600	< 3.55

LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

The following blood components do not have a significant effect (+/- 20 % of expected) on the test results up to the concentrations stated below:

Hemoglobin	2.0 mg/mL
Bilirubin	1.0 mg/mL
Triglyceride	91 mg/mL

PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Adrenaline	Noradrenaline	Cross-reactivity of other substances tested < 0.01 %	
	Adrenaline	100	< 0.02		
	Noradrenaline	0.23	100		
	Metanephrine	<0.005	< 0.002		
	Normetanephrine	<0.001	<0.005		
Analytical Sensitivity (Limit of Detection)	Adrenaline	Urine	0.3 ng/mL	Mean signal (Zero-Standard) + 2SD	
		Plasma	10 pg/mL		
	Noradrenaline	Urine	0.6 ng/mL		
		Plasma	20 pg/mL		
Precision			Range (ng/mL)	CV (%)	
Intra-Assay	Adrenaline	Urine	5.9 – 81.2	5.4	
		Plasma	0.057 – 0.837	7.1	
	Noradrenaline	Urine	16.0 – 256	7.3	
		Plasma	0.560 – 12.38	7.4	
Inter-Assay	Adrenaline	Urine	6.4 – 89.3	10.1	
		Plasma	0.106 – 1.064	13.5	
	Noradrenaline	Urine	15.4 – 391.5	12.1	
		Plasma	0.569 – 1.945	12.5	
Linearity			Range (ng/mL)	Serial dilution up to	Range (%)
	Adrenaline	Urine	23.6 – 90.3	1:32	86 - 125
		Plasma	1.4 – 5.3	1:32	79 – 126

	Noradrenaline	Urine	178 – 423	1:32	85 - 115
		Plasma	0.64 – 8.23	1:32	89 - 111
	No High dose hook effect detected.				
Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Adrenaline	Urine	95.0	85.6 – 108.0	
		Plasma	99.0	75.0 – 109.0	
	Noradrenaline	Urine	100.9	81 – 116	
		Plasma	97.5	83 - 111	
Method Comparison versus HPLC	Adrenaline	DRG= 0.906 x HPLC + 13.9; r = 0.969; n = 120			
	Noradrenaline	DRG = 0.75 x HPLC + 4.8; r = 0.945; n = 134			

PRODUCT LITERATURE REFERENCES

1. Rust MB, Faulhaber J et. al. Neurogenic Mechanisms Contribute to Hypertension in Mice with Disruption of the K-CL Cotransporter KCC3. Circulation Research, January (2006)
2. Creces J., Appleton Ch.: Catecholamines and their Metabolites: Evaluation of a commercial ELISA. Clin. Biochem., QML Pathology, Brisbane QLD (2004)
3. Adams, J. M. et al. Effects of 17β-Estradiol on hypoglycemia-induced increases in plasma catecholamines in the rat. Poster Society for Neuroscience, Annual Meeting, New Orleans (2003)
4. Westermann J, Hubl W, Kaiser N, Salewski L, Simple, rapid and sensitive determination of epinephrine and norepinephrine in urine and plasma by non-competitive enzyme immunoassay, compared with HPLC method. Clin. Lab., 48: 61-71 (2002)

DRG® CatCombi ELISA (EIA-3175)

REVISED 31 JULY 2009 (VERS. 4.0)

RUO IN THE USA

SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..