DRG® Cat Combi (Plasma) Ultrasensitive Elisa (EIA-5131)

USA: RUO

As of 22 Feb. 2010 rm (Vers. 1.0)

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

1 INTENDED USE AND PRINCIPLE OF THE TEST

High Sensitive Enzyme Immunoassay for the quantitative determination of Adrenaline (Epinephrine), and Noradrenaline (Norepinephrine) in plasma

Adrenaline (epinephrine) and noradrenaline (norepinephrine) are 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

2 ADVICE ON HANDLING THE TEST

2.1 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, 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.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

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 and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. 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 safety data sheets of the individual products are available upon request. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 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 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.

2.6 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.

3 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.

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4 CONTENTS OF THE KIT

96	**Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart
FOILS	**Adhesive Foil**	2 x 4	ready for use
WASH-CONC 50x	**Wash Buffer Concentrate**	2 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
CONJUGATE	**Enzyme Conjugate**	2 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
SUBSTRATE	**Substrate**	2 x 12 mL	ready for use, containing a solution of TMB
STOP-SOLN	**Stop Solution**	2 x 12 mL	ready for use, containing 0.25 M H_2SO_4
ARD MN	**Adrenaline-Metanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
NAD NMN	**Noradrenaline-Normetanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
ADR-AS	**Adrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
NAD-AS	**Noradrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
STANDARD A	**Standard A**	1 x 6 mL	ready for use
STANDARD B	**Standard B**	1 x 6 mL	ready for use
STANDARD C	**Standard C**	1 x 6 mL	ready for use
STANDARD D	**Standard D**	1 x 6 mL	ready for use
STANDARD E	**Standard E**	1 x 6 mL	ready for use
STANDARD F	**Standard F**	1 x 6 mL	ready for use
EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	coated with boronate affinity gel
HCL	**Hydrochloric Acid**	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
CONTROL 1	**Control 1**	1 x 6 mL	ready for use
CONTROL 2	**Control 2**	1 x 6 mL	ready for use
ACYL-CONC	**Acylation Concentrate**	1 x 0.5 mL	Concentrate. Has to be diluted prior to use.
ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use

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ACYL-DILUENT	**Acylation Diluent**	1x 4 mL	ready for use
ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
ASSAY-BUFF	**Assay Buffer**	2 x 4 mL	ready for use, contains 1 M HCl
COENZYME	**Coenzyme**	1 x 2 mL	ready for use, S-adenosyl-L-methionine
ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains COMT
EXTRACT-BUFF	**Extraction Buffer**	2 x 4 mL	ready for use

4.1 Additional materials and equipment required but not provided in the kit

Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000µL)
Microtiter plate washing device
ELISA reader capable of reading absorbance at 450 nm (reference filter 620 – 650 nm)
Shaker (shaking amplitude 3mm; approx. 600 rpm)
Absorbent material (paper towel)
Distilled water
Vortex mixer

5 SAMPLE COLLECTION AND STORAGE

Plasma

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.
Repeated freezing and thawing should be avoided.

6 TEST PROCEDURE

Allow reagents and samples to reach room temperature. Duplicate measurements are recommended.

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2–8°C

Acylation Solution

The Acylation Concentrate has to be diluted 1 + 60 with Acylation-Diluent in a glass or polypropylene-vial.

Acylation Concentrate	10 µL	20 µL	25 µL	50 µL
Acylation-Diluent	600 µL	1.2 mL	1.5 mL	3 mL

The Acylation Solution has to be prepared freshly prior to the assay (not longer than 60 minutes in advance). Discard after use!

Enzyme Solution

Reconstitute the content of the vial labelled '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!

6.2 Extraction and Acylation

1. Pipette **750 µL** of **Standards**, **controls** and **plasma sample** into the respective wells of the Extraction Plate.

   **** If only a plasma volume < 750 µl is available add dist. water to the plasma sample, to a final volume of 750 µl.***
2. Pipette **50 µL** of **Assay Buffer** into all wells.
3. Pipette **50 µL** of **Extraction Buffer** into all wells
4. Cover the plate with adhesive foil. Shake **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
5. Empty the plate. Blot dry by tapping the inverted plate on absorbent material.
6. Pipette **1 mL** of **Wash Buffer** into all wells.
7. Shake **5 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8. Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.
9. **Wash one more time** as described (step 6, 7 and 8)!
10. Pipette **150 µL** of **Acylation Buffer** into all wells.
11. Pipette **25 µL** of **Acylation Solution** (refer to 6.1*)* into all wells.
12. Shake **20 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
13. Empty the plate and blot dry by tapping the inverted plate on absorbent material.
14. Pipette **1 mL** of **Wash Buffer** into all wells.
15. Shake **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
16. Empty the plate. Blot dry by tapping the inverted plate on absorbent material.
17. **Wash one more time** as described (step 14, 15, 16).

18. Pipette **200 µL** of **Hydrochloric Acid** into all wells.
19. Cover plate with adhesive foil. Shake **10 min** at **RT** (20-25°C) on an o shaker (approx. 600 rpm).

Do not decant the supernatant thereafter!

190 µL of the supernatant is needed for the subsequent enzymatic conversion

6.3 Enzymatic Conversion

1. Pipette **190 µL** of the **extracted standards, controls** and **samples** into the respective wells of the **Microtiter Plate.**
2. Add **50 µL** of **Enzyme Solution** (refer to 6.1*)* to all wells.
3. Cover plate with **Adhesive Foil**. Shake **1 min** at **RT (20-25°C)** on a shaker to mix.
4. Incubate for **2 hours** at **37°C**. The following volumes of the supernatants are needed for the subsequent ELISA:

Adrenaline	**100 µL**	**Noradrenaline**	**25 µL**

6.4 ELISA

Adrenaline ELISA

1. Pipette **100 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Adrenaline Microtiter Strips**.
2. Pipette **50 µL** of the respective **Adrenaline Antiserum** into all wells.
3. Cover the plate with **Adhesive Foil**. Incubate for **1 min** at **RT** (20-25°C) on a **shaker**.
4. Incubate for **15 – 20 hours** (overnight) at **2 – 8 °C.**
5. Remove the foil and discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with 300 µL **Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
6. Pipette **100 µL** of **Enzyme Conjugate** into all wells.
7. Incubate **30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8. Discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with 300 µl **Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
9. Pipette **100 µL** of **Substrate** into all wells.
10. Incubate **20-30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).

Avoid exposure to direct sun light!

11. Pipette **100 µL** of **Stop Solution** into all wells.
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.

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Noradrenaline ELISA

1. Pipette **25 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Noradrenaline Microtiter Strips**.
2. Pipette **50 µL** of the respective **Noradrenaline Antiserum** into all wells.
3. Cover the plate with **Adhesive Foil**. Incubate for **1 min** at **RT** (20-25°C) on a **shaker**.
4. Incubate for **15 – 20 hours** (overnight) at **2 – 8 °C.**
5. Remove the foil and discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with 300 µL **Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
6. Pipette **100 µL** of **Enzyme Conjugate** into all wells.
7. Incubate **30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8. Discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with 300 µl **Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
9. Pipette **100 µL** of **Substrate** into all wells.
10. Incubate **20-30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).

Avoid exposure to direct sun light!

11. Pipette **100 µL** of **Stop Solution** into all wells.
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

The calibration curve from which the concentrations of the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Adrenaline (pg/mL)	0	15	50	150	500	1 500
Adrenaline (pmol/L)	0	81.9	273	819	2 730	8 190
Noradrenaline (pg/mL)	0	60	200	600	2 000	6 000
Noradrenaline (pmol/L)	0	354.6	1 182	3 546	11 820	35 460
Conversion:	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L)					

The concentrations of the **plasma samples** and the **controls** can be read directly from the standard curve.

If only a plasma volume < 750 µl is available, the concentration of the sample have to be **multiplied with a volume-factor.**

$$\textbf{Volume-factor} = \frac{\textbf{750}}{\textbf{available volume}}$$

7.1 Quality control

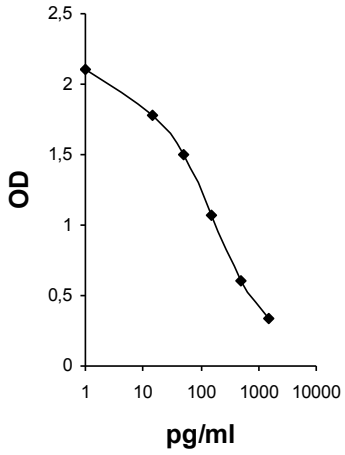
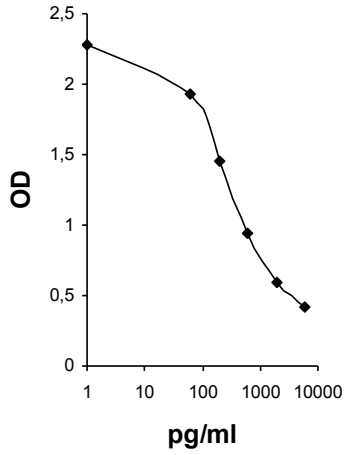
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

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. 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

7.3 Typical calibration curves

ADRENALINE	NORADRENALINE
	
Example. Do not use for calculation!	*Example. Do not use for calculation!*

8 ASSAY CHARACTERISTICS

Expected Reference Values		Adrenaline	Noradrenaline
	Plasma	< 100 pg/mL	< 600 pg/mL

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD		
		Adrenaline	Noradrenaline
	Plasma	5 pg/mL	20 pg/mL

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Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**	
		Noradrenaline	Adrenaline
	Derivatized Adrenaline	0.14	100
	Derivatized Noradrenaline	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 Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Adrenaline	high	1329.3 ± 372.6	124.2	9.3
	medium	412.1 ± 129.6	43.2	10.5
	low	37.9 ± 19.5	6.5	17.1
Noradrenaline	high	1377.4 ± 483.6	161.2	11.7
	medium	502.6 ± 126.9	42.3	8.4
	low	32.7 ± 15.3	5.1	15.6

Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Adrenaline				
Human EDTA-Plasma	104.0	89.4 – 128.3	13.1	12.6
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 – 125.6	8.0	6.9

For actual literature, information about clinical significance or any other information please contact your local supplier.

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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 tilstrækkeligt 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	Ογκος/αριθ..