DRG® TriCat Research ELISA (EIA-4891)

As of 1 Aug. 2008 (Vers. 1.0)

RUO

PRINCIPLE OF THE TEST

Adrenaline (Epinephrine), Noradrenaline (Norepinephrine) and Dopamine 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.

STORAGE AND STABILITY

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date shown on the kit labels.

CONTENTS OF THE KIT

Acylation Buffer	1 x 20 mL	ready for use
Assay Buffer	2 x 4 mL	ready for use, contains 1 M HCl
Coenzyme	2 x 0.75 mL	ready for use, S-adenosyl-L-methionine
Enzyme	4 x 1 mL	lyophilized, contains COMT
Extraction Buffer	2 x 4 mL	ready for use
Extraction Plate	2 x 48 wells	coated with boronate affinity gel
Hydrochloric Acid	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
Adjustment Buffer	1 x 4 mL	ready for use
Acylation Diluent	1x 4 mL	ready for use
Standard A	1 x 1 mL	ready for use
Standard B	1 x 1 mL	ready for use
Standard C	1 x 1 mL	ready for use
Standard D	1 x 1 mL	ready for use
Standard E	1 x 1 mL	ready for use
Standard F	1 x 1 mL	ready for use
Acylation Concentrate	1 x 0.25 mL	Concentrate. Has to be diluted prior to use.
Control 1	1 x 1 mL	ready for use
Control 2	1 x 1 mL	ready for use
Wash Buffer Concentrate 25X	3 x 20 mL	Concentrate. Dilute content with distilled water to a final volume of 500 mL
Enzyme Conjugate	3 x 11 mL	ready for use, anti-rabbit IgG conjugated with peroxidase

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Substrate	3 x 11 mL	ready for use, containing a solution of TMB
Stop Solution	3 x 11 mL	ready for use, containing 0.25 M H_2SO_4
Adhesive Foil	3 x 4	ready for use
Adrenaline-Metanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
Noradrenaline-Normetanephrine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
Dopamine Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, green coloured
Microtiter Plate	1 x 96 wells	12 strips, 8 wells each, break apart
Adrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
Noradrenaline Antiserum	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
Dopamine Antiserum	1 x 6 mL	from rabbit, ready for use, green coloured, green screw cap

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

SAMPLE COLLECTION AND STORAGE

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 1mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

TEST PROCEDURE

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

1.1 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.

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!*

1.2 Sample preparation

The Catecholamine Research 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 catecholamine degradation by adding preservatives to the sample (see 5. Sample Collection and Storage).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Adrenaline, Noradrenaline and Dopamine. If your samples already contain high amounts of perchloric acid, neutralize them prior to the extraction step.
- Tissue samples can be homogenised in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions, Adrenaline, Noradrenaline and Dopamine are 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 catecholamines.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the catecholamines in your samples. Prepare a stock solution of Adrenaline, Noradrenaline and Dopamine. 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 catecholamines in your sample by testing different amounts of sample volume.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer or your local distributor directly!

1.3 Extraction and Acylation

The TriCat Research ELISA offers a flexible test system for various biological sample types and volumes. Step 1 of the extraction procedure depends on the sample volume:

- in case you have sample volumes between 1 – 100 µL follow **1.1**
- in case you have sample volumes between 100 – 500 µL follow **1.2**
- in case you have sample volumes between 500 – 750 µL follow **1.3**

⚠ ***Within a run it is only possible to measure samples with the same volume!***

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1.	1.1	1.2	1.3
	Sample volume 1 – 100 µL	**Sample volume 100 – 500 µL**	**Sample volume 500 – 750 µL**
	Pipette into the respective wells of the Extraction Plate:	Pipette into the respective wells of the Extraction Plate:	Pipette into the respective wells of the Extraction Plate:
	30 µL standards, 30 µL controls and 1 – 100 µL of the sample.	**30 µL standards, 30 µL controls and 100 – 500 µL of the sample**.	**30 µL of Standards, 30 µL of controls and 500 – 750 µL of sample**.
	Fill up each well with distilled water to a **final volume** of 100 µL (e.g. 30 µL standard plus 70 µL dist. water).	Fill up each well with distilled water to a **final volume** of 500 µL (e.g. 30 µL standard plus 470 µL dist. water).	Fill up each well with distilled water to a **final volume** of 750 µL (e.g. 30 µL standard plus 720 µL dist. water).

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. Incubate **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).

5. Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.

6. Pipette **1 mL** of **Wash Buffer** into all wells. Cover the plate with adhesive foil.

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. Incubate **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. Cover plate with adhesive foil.

15. Shake **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).

16. Remove the foil and 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. Incubate **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

1.4 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**. Incubate **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 75 µL **Noradrenaline 75 µL** **Dopamine 75 µL**

1.5 Adrenaline, Noradrenaline and Dopamine ELISA

1. Pipette **75 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Mikrotiter Strips (*1)**.
2. Pipette **50 µL** of the respective **Antiserum (*2)** 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. Cover the plate with **Adhesive Foil** and incubate **30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8. 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.
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.

⚠ (*1): **Adrenaline Microtiter Strips, Noradrenaline Microtiter Strips, Dopamine Microtiter Strips**

(*[2]): **Adrenaline Antiserum, Noradrenaline Antiserum, Dopamine Antiserum**

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.

The standards refer to:

	Concentration of the standards (ng/mL)					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Adrenaline/Noradrenaline	0	0.45	1.5	4.5	15	45
Dopamine	0	0.9	3	9	30	90

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

$$\textbf{Correction factor} = \frac{\textbf{30 µL (volume of standards extracted)}}{\textbf{sample volume (µL) extracted}}$$

Example: 750µL of the sample is extracted and the concentration taken from the standard curve is 0.45 ng/mL Noradrenaline.

Correction factor = 30/750 = 0.04

Concentration of the sample = 0.45 ng/mL x 0.04 = 0.018ng/mL = 18 pg/mL Noradrenaline

1.6 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.

1.7 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*

ASSAY CHARACTERISTICS

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)		
		Noradrenaline	Adrenaline	Dopamine
	Derivatized Adrenaline	0.14	100	0.03
	Derivatized Noradrenaline	100	0.20	0.87
	Derivatized Dopamine	0.2	< 0.0007	100
	Metanephrine	< 0.003	0.64	< 0.007
	Normetanephrine	0.48	0.0009	0.008
	3-Methoxytyramine	< 0.003	< 0.0007	0.55
	3-Methoxy-4-hydroxyphenylglycol	0.01	0.03	< 0.007
	Tyramine	< 0.003	< 0.0007	0.13
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003	< 0.0007	< 0.007

Analytical Sensitivity (Limit of Detection)	Adrenaline	Noradrenaline	Dopamine
	0.3 ng/mL x C*	0.2 ng/mL x C*	0.7 ng/mL x C*

C* = Correction factor (refer to 7.)

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
Dopamine	high	1438.6 ± 465.6	155.2	10.8
	medium	565.9 ± 246.3	82.1	14.5
	low	56.4 ± 36.3	12.1	21.5
Intra-Assay Cell Culture Medium (RPMI)				
	Sample	*Mean ± 3 SD (pg/mL)*	*SD (pg/mL)*	*CV (%)*
Adrenaline	high	1649.6 ± 555.0	185	11.2
	medium	526.2 ± 186.6	62.2	11.8

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	low	38.7 ± 18.9	6.3	16.3
Noradrenaline	high	2027.8 ± 712.5	237.5	11.7
	medium	716.5 ± 179.7	59.9	8.4
	low	46.0 ± 16.8	5.6	12.2
Dopamine	high	2784.5 ± 1238.7	412.9	14.8
	medium	1003.7 ± 526.2	175.4	17.5
	low	74.7 ± 51.6	17.2	23.0

Recovery	*Mean (%)*	*Range (%)*	*SD (%)*	*CV (%)*
Adrenaline				
Human EDTA-Plasma	104.0	89.4 – 128.3	13.1	12.6
Cell Culture Medium	95.5	81.6 – 109.6	8.3	8.7
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 – 125.6	8.0	6.9
Cell Culture Medium	96.7	70.6 – 124.7	17.1	17.7
Dopamine				
Human EDTA-Plasma	97.7	83.7 – 115.9	11.8	12.1
Cell Culture Medium	98.6	77.7 – 113.4	12.1	12.2

ADVICE ON HANDLING THE TEST

1.8 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.

1.9 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 complaint form and return it completely filled in to the manufacturer.

1.10 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.

1.11 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.

1.12 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.

1.13 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.

⚠ ***For actual literature, information about clinical significance or any other information about the test contact the manufacturer directly.***

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SYMBOLS USED WITH DRG ASSAY´S

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

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	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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