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Instructions for use **2-CAT ELISA**

For Instructional/Research Purposes Only

REF

IB89549R



RUO

For research
use only –
Not for use
in diagnostic
procedures

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of adrenaline (epinephrine) and noradrenaline (norepinephrine) in plasma and urine.

Adrenaline (epinephrine) and noradrenaline (norepinephrine) are extracted by using a cis-diol-specific affinity gel, acylated and then converted 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 antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody 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 standard curve prepared with known standard concentrations.

1.2 Background

In humans the catecholamines adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine are neurotransmitters of the sympathetic nervous system and are involved in many physiological processes. The sympathetic nervous system sets the body to a heightened state of alert, also called as the body's fight-or-flight response.

In the human body the catecholamines and their metabolites indicate the adaption of the body to acute and chronic stress.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

- (1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- (2) The principles of Good Laboratory Practice (GLP) have to be followed.
- (3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (6) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
- (7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- (9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- (10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- (11) A standard curve must be established for each run.
- (12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
- (13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- (14) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (15) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- (16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of catecholamine level in the sample.

2.2.3 High-Dose-Hook effect


No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

BA D-0090	FOILS	Adhesive Foil - Ready to use
Content:	Adhesive Foils in a resealable pouch	
Volume:	2 x 4 foils	
BA E-0030	WASH-CONC 50x	Wash Buffer Concentrate - Concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	2 x 20 ml/vial, light purple cap	
BA E-0040	CONJUGATE	Enzyme Conjugate - Ready to use
Content:	Goat anti-rabbit immunoglobulins, conjugated with peroxidase	
Volume:	2 x 12 ml/vial, red cap	
BA E-0055	SUBSTRATE	Substrate - Ready to use
Content:	Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	2 x 12 ml/vial, black cap	
BA E-0080	STOP-SOLN	Stop Solution - Ready to use
Content:	0.25 M sulfuric acid	
Volume:	2 x 12 ml/vial, light grey cap	
Hazards identification:		H290 May be corrosive to metals.
BA E-0131	ADR MN	Adrenaline Microtiter Strips - Ready to use
Content:	1 x 96 well (12x8) antigen precoated microwell plate in a resealable blue pouch with desiccant	
BA E-0231	NAD NMN	Noradrenaline Microtiter Strips - Ready to use
Content:	1 x 96 well (12x8) antigen precoated microwell plate in a resealable yellow pouch with desiccant	

BA E-6110 **ADR-AS** **Adrenaline Antiserum** - Ready to use

Content: Rabbit anti-adrenaline antibody, blue coloured

Volume: 1 x 6 ml/vial, blue cap

BA E-6210 **NAD-AS** **Noradrenaline Antiserum** - Ready to use

Content: Rabbit anti-noradrenaline antibody, yellow coloured

Volume: 1 x 6 ml/vial, yellow cap

BA R-0050 **ADJUST-BUFF** **Adjustment Buffer** - Ready to use

Content: TRIS buffer

Volume: 1 x 4 ml/vial, green cap

Standards and Controls - Ready to use

Cat. no.	Component	Colour/ Cap	Concentration ng/ml		Concentration nmol/l		Volume/ Vial
			ADR	NAD	ADR	NAD	
BA E-6601	STANDARD A	white	0	0	0	0	4 ml
BA E-6602	STANDARD B	light yellow	1	5	5.5	30	4 ml
BA E-6603	STANDARD C	orange	4	20	22	118	4 ml
BA E-6604	STANDARD D	dark blue	15	75	82	443	4 ml
BA E-6605	STANDARD E	light grey	50	250	273	1 478	4 ml
BA E-6606	STANDARD F	black	200	1 000	1 092	5 910	4 ml
BA E-6651	CONTROL 1	light green	Refer to QC report for expected value and				4 ml
BA E-6652	CONTROL 2	dark red	acceptable range!				4 ml

Conversion: Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l)

Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/l)

Content: Acidic buffer with non-mercury stabilizer, spiked with defined quantity of adrenaline and noradrenaline

BA R-6611 **ACYL-BUFF** **Acylation Buffer** - Ready to use

Content: Buffer with light alkaline pH for the acylation

Volume: 1 x 20 ml/vial, white cap

BA R-6612 **ACYL-REAG** **Acylation Reagent** - Ready to use

Content: Acylation reagent in DMF and DMSO

Volume: 1 x 3 ml/vial, light red cap

Hazards identification:



H360D May damage the unborn child.

H226 Flammable liquid and vapour.

H312 + H332 Harmful in contact with skin or if inhaled.

H319 Causes serious eye irritation.

BA R-6613 **ASSAY-BUFF** **Assay Buffer** - Ready to use

Content: 1M hydrochloric acid and a non-mercury preservative

Volume: 1 x 6 ml/vial, light grey cap

BA R-6614 **COENZYME** **Coenzyme** - Ready to use

Content: S-adenosyl-L-methionine

Volume: 1 x 4 ml/vial, purple cap

BA R-6615 **ENZYME** **Enzyme** - Lyophilized

Content: Catechol-O-methyltransferase

Volume: 4 vials, pink cap

BA R-6617 **EXTRACT-BUFF** **Extraction Buffer** - Ready to use

Content: Buffer containing carbonate

Volume: 1 x 6 ml/vial, brown cap

BA R-6618 **EXTRACT-PLATE 48** **Extraction Plate** - Ready to use

Content: 2 x 48 well plates coated with boronate affinity gel in a resealable pouch

BA R-6619 **HCL** **Hydrochloric Acid** - Ready to use

Content: 0.025 M Hydrochloric Acid, yellow coloured

Volume: 1 x 20 ml/vial, dark green cap

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

- Calibrated precision pipettes to dispense volumes between 10 – 700 µl; 1 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

5. Sample collection and storage

Plasma

Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™ for plasma) and centrifuged according to manufacturer's instructions immediately after collection. Haemolytic and lipemic samples should not be used for the assay.

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

Repeated freezing and thawing should be avoided.

Urine

Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.

If 24-hour urine is used please record the total volume of the collected urine.


Storage: up to 48 hours at 2 - 8 °C, up to 24 hours at room temperature, for longer periods (up to 6 month) 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. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the absorbance may vary if a thermostat is not used. The higher the temperature, the higher the absorbance will be. Varying incubation times will have a similar influence on the absorbance. 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*

6.1 Preparation of reagents


Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 2 – 8 °C

Enzyme Solution


Reconstitute the content of the vial labelled 'Enzyme' with 1 ml water (deionized, distilled, or ultra-pure) 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!*


Adrenaline Microtiter Strips and Noradrenaline Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

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 water (deionized, distilled, or ultra-pure) 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 and discard.				
	Do not decant the supernatant thereafter!				
	The following volumes of the supernatant are needed for the subsequent ELISA:				
	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; text-align: center;">Adrenaline</td> <td style="width: 50%; text-align: center;">100 µl</td> <td style="width: 50%; text-align: center;">Noradrenaline</td> <td style="width: 50%; text-align: center;">20 µl</td> </tr> </table>	Adrenaline	100 µl	Noradrenaline	20 µl
Adrenaline	100 µl	Noradrenaline	20 µl		

6.3 Adrenaline ELISA

1.	Pipette 25 µl of the Enzyme Solution (refer to 6.1) into all wells of the Adrenaline Microtiter Strips .
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 respective Adrenaline Antiserum into all wells and cover plate with Adhesive Foil .
5.	Incubate for 2 h at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
6.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time 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. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
10.	Pipette 100 µl of the Substrate into all wells and incubate for 25 ± 5 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).  Avoid exposure to direct sunlight!
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 (if available a reference wavelength between 620 nm and 650 nm is recommended).

6.4 Noradrenaline ELISA

1. Pipette 25 µl of the Enzyme Solution (refer to 6.1) into all wells of the Noradrenaline Microtiter Strips .
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 Noradrenaline Antiserum into all wells and cover plate with Adhesive Foil .
5. Incubate for 2 h at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
6. Remove the foil. Discard or aspirate the content of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time 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. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
10. Pipette 100 µl of the Substrate into all wells and incubate for 25 ± 5 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). ⚠ Avoid exposure to direct sunlight!
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 (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range		Adrenaline	Noradrenaline
	Urine		0.7 - 200 ng/ml
Plasma		18 - 6667 pg/ml	93 - 33 333 pg/ml

The standard curves are obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

Urine samples and controls

The concentrations of the **urine samples** and the **Controls** can be read directly from the standard curve. Calculate the 24 h excretion for each urine sample: **µg/24h = µg/l x l/24h**

Plasma samples

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

Conversion

Adrenaline (ng/ml) x 5.46 = Adrenaline (nmol/l)

Noradrenaline (ng/ml) x 5.91 = Noradrenaline (nmol/l)

Expected reference values


It is strongly recommended that each laboratory should determine its own normal and abnormal values.

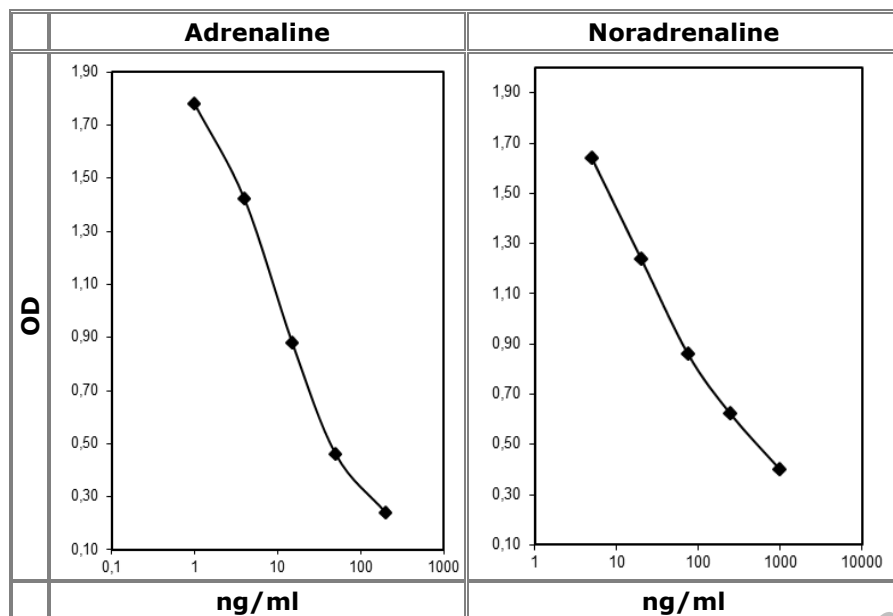
	Adrenaline	Noradrenaline
24-hour urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)
Plasma	< 100 pg/ml	< 600 pg/ml

7.1 Quality control

The confidence limits of the kit controls are printed on the QC-Report.

7.2 Typical standard curves

 Examples, do not use for calculation!



8. Assay characteristics

Analytical Sensitivity	LOD	Urine (ng/ml)	Adrenaline	Noradrenaline
		Plasma (pg/ml)	0.9	1.7
	LOQ	Urine (ng/ml)	10	36
		Plasma (pg/ml)	0.7	2.5
		18	93	

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	
		Adrenaline	Noradrenaline
	Derivatized Adrenaline	100	0.08
	Derivatized Noradrenaline	0.13	100
	Derivatized Dopamine	< 0.01	0.03
	Metanephrine	0.18	< 0.01
	Normetanephrine	< 0.01	0.16
	3-Methoxytyramine	< 0.01	< 0.01
	3-Methoxy-4-hydroxyphenylglycol	< 0.01	< 0.01
	Tyramine	< 0.01	< 0.01
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.01	< 0.01

Precision							
Intra-Assay Urine (n = 60)				Intra-Assay Plasma (n = 60)			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (pg/ml)	CV (%)
Adrenaline	1	6.2 ± 1.1	17.4	Adrenaline	1	64.7 ± 15.9	24.7
	2	21.4 ± 2.7	12.4		2	258 ± 32.5	12.7
	3	59.4 ± 7.8	13.1		3	948 ± 105	11.0
Noradrenaline	1	26.1 ± 3.6	13.8	Noradrenaline	1	510 ± 65	12.8
	2	97 ± 12.8	13.4		2	1358 ± 194	14.3
	3	267 ± 35	13.1		3	3363 ± 374	11.1

Inter-Assay Urine (n = 33)				Inter-Assay Plasma (n = 18)			
	Sample	Range (ng/ml)	CV (%)		Sample	Range (pg/ml)	CV (%)
Adrenaline	1	5.2 ± 0.9	17.9	Adrenaline	1	76.4 ± 11.1	14.5
	2	17.8 ± 2.1	11.7		2	247 ± 27.5	11.1
	3	54.2 ± 6.6	12.1		3	771 ± 101	13.1
Noradrenaline	1	19.5 ± 3.9	20.0	Noradrenaline	1	445 ± 40.9	9.2
	2	80.6 ± 10.6	13.2		2	1232 ± 134	10.9
	3	226 ± 39.5	17.4		3	3283 ± 302	9.2

Linearity			Serial dilution up to	Range (%)	Mean (%)
	Adrenaline	Urine	1:512	92 - 123	108
		Plasma	1:512	94 - 115	105
	Noradrenaline	Urine	1:512	100 - 127	112
		Plasma	1:512	102 - 125	112







Recovery			Mean (%)	Range (%)	Range
	Adrenaline	Urine	106	94 - 120	4.5 - 53.5 ng/ml
		Plasma	105	88 - 117	9.1 - 4268 pg/ml
	Noradrenaline	Urine	103	91 - 113	58.6 - 260 ng/ml
		Plasma	87	75 - 107	51 - 14 251 pg/ml

9. References/Literature

- (1) Kim et al. Vitamin C prevents stress-induced damage on the heart caused by the death of cardiomyocytes, through the down-regulation of the excessive production of catecholamine, TNF- α , and ROS production in GULO(-I-) Vit C-Insufficient mice. Free Radical Biology and Medicine, 65:573-583 (2013)
- (2) Bada et al. Peripheral vasodilatation determines cardiac output in exercising humans: insight from atrial pacing. The Journal of Physiology, 590(8):2051-2060 (2012)
- (3) Parks et al. Employment and work schedule are related to telomere length in women. Occupational & Environmental Medicine 68(8):582-589 (2011)

△ For updated literature or any other information please contact your local supplier.

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!