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**Instructions for use**

**3-CAT ELISA**

**(Adrenaline – Noradrenaline - Dopamine)**

Enzyme Immunoassay for the determination  
of Adrenaline (Epinephrine), Noradrenaline (Norepinephrine),  
and Dopamine in plasma and urine

For Research use only-  
Not for use in diagnostic procedures

**REF** **IB89520**



**RUO**

### 1. 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. The determination of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

### 2. Storage and stability

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

### 3. Contents of the kit

<b>BA 1611</b>	ACYL-BUFF	<b>Acylation Buffer</b>	1 x 20 mL	ready for use
<b>BA 1612</b>	ACYL-REAG	<b>Acylation Reagent</b>	2 x 1.5mL	ready for use
<b>BA 1613</b>	ASSAY-BUFF	<b>Assay Buffer</b>	2 x 4 mL	ready for use, contains 1 M HCl
<b>BA 1614</b>	COENZYME	<b>Coenzyme</b>	3 x 0.75 mL	ready for use, S-adenosyl-L-methionine
<b>BA 1615</b>	ENZYME	<b>Enzyme</b>	6 x 1 mL	lyophilized, contains the enzyme catechol-O-methyltransferase
<b>BA 1617</b>	EXTRACT-BUFF	<b>Extraction Buffer</b>	2 x 4 mL	ready for use
<b>BA 1618</b>	EXTRACT-PLATE 48	<b>Extraction Plate</b>	2 x 48 wells	coated with boronate affinity gel
<b>BA 1619</b>	HCL	<b>Hydrochloric Acid</b>	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl
<b>BA 3050</b>	ADJUST-BUFF	<b>Adjustment Buffer</b>	2 x 4 mL	ready for use
<b>BA 10-0025</b>	WASH-CONC 25x	<b>Wash Buffer Concentrate</b>	3 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 500 mL
<b>BA 10-0040</b>	CONJUGATE	<b>Enzyme Conjugate</b>	3 x 11 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
<b>BA 10-0055</b>	SUBSTRATE	<b>Substrate</b>	3 x 11 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
<b>BA 10-0080</b>	STOP-SOLN	<b>Stop Solution</b>	3 x 11 mL	ready for use, containing 0.25 M H <sub>2</sub> SO <sub>4</sub>
<b>BA 10-0090</b>	FOILS	<b>Adhesive Foil</b>	3 x 4	ready for use
<b>BA 10-0110</b>	ADR-AS	<b>Adrenaline Antiserum</b>	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
<b>BA 10-0131</b>	ADR MN	<b>Adrenaline-Metanephrine Microtiter Strips</b>	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, blue coloured
<b>BA 10-0210</b>	NAD-AS	<b>Noradrenaline Antiserum</b>	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
<b>BA 10-0231</b>	NAD NMN	<b>Noradrenaline-Normetanephrine Microtiter Strips</b>	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
<b>BA 10-0310</b>	DOP-AS	<b>Dopamine Antiserum</b>	1 x 6 mL	from rabbit, ready for use, green coloured, green screw cap
<b>BA 10-0331</b>	DOP	<b>Dopamine Microtiter Strips</b>	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, green coloured
<b>BA 10-1601</b>	STANDARD A	<b>Standard A</b>	1 x 1 mL	ready for use
<b>BA 10-1602</b>	STANDARD B	<b>Standard B</b>	1 x 1 mL	ready for use
<b>BA 10-1603</b>	STANDARD C	<b>Standard C</b>	1 x 1 mL	ready for use
<b>BA 10-1604</b>	STANDARD D	<b>Standard D</b>	1 x 1 mL	ready for use
<b>BA 10-1605</b>	STANDARD E	<b>Standard E</b>	1 x 1 mL	ready for use
<b>BA 10-1606</b>	STANDARD F	<b>Standard F</b>	1 x 1 mL	ready for use
<b>BA 10-1609</b>	STANDARD A/B	<b>Standard A/B*</b>	1 x 1 mL	ready for use
<b>BA 10-1651</b>	CONTROL 1	<b>Control 1</b>	1 x 1 mL	ready for use
<b>BA 10-1652</b>	CONTROL 2	<b>Control 2</b>	1 x 1 mL	ready for use

⚠ \* for the determination of dopamine in plasma the additional **Standard A/B** is mandatory!

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

- Calibrated variable precision micropipettes (e.g. 10-100  $\mu\text{L}$  / 100-1.000 $\mu\text{L}$ )
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 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.

##### Urine

Spontaneous or 24-hours urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used.

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

Repeated freezing and thawing should be avoided.

Avoid exposure to direct sunlight.

#### 6. Test procedure

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

##### 6.1 Preparation of reagents

###### Wash Buffer

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

###### Enzyme Solution

Reconstitute the content of the vial 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 Sample preparation, extraction and acylation

⚠ *For the determination of dopamine in plasma the additional **Standard A/B** is mandatory!*

1.	Pipette <b>20 <math>\mu\text{L}</math></b> of <b>standards, controls, urine samples</b> and <b>600 <math>\mu\text{L}</math></b> of <b>plasma samples</b> into the respective wells of the <b>Extraction Plate</b> .
2.	Add <b>500 <math>\mu\text{L}</math></b> of <b>distilled water</b> to the wells with <b>standards, controls</b> and <b>urine samples</b> .
3.	Pipette <b>50 <math>\mu\text{L}</math></b> of <b>Assay Buffer</b> into all wells
4.	Pipette <b>50 <math>\mu\text{L}</math></b> of <b>Extraction Buffer</b> into all wells
5.	Cover plate with adhesive foil and incubate <b>30 min</b> at <b>RT</b> (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 <b>1 mL</b> of <b>Wash Buffer</b> into all wells. Incubate the plate for <b>5 min</b> at <b>RT</b> (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 <b>1 mL</b> of <b>Wash Buffer</b> into all wells. Incubate the plate for <b>5 min</b> at <b>RT</b> (20-25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
9.	Pipette <b>150 <math>\mu\text{L}</math></b> of <b>Acylation Buffer</b> into all wells.
10.	Pipette <b>25 <math>\mu\text{L}</math></b> of <b>Acylation Reagent</b> into all wells.
11.	Incubate <b>15 min</b> at <b>RT</b> (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 <b>1 mL</b> of <b>Wash Buffer</b> into all wells. Incubate the plate for <b>10 min</b> at <b>RT</b> (20-25°C) on a shaker (approx. 600 rpm). Empty plate and blot dry by tapping the inverted plate on absorbent material.
14.	Pipette <b>200 <math>\mu\text{L}</math></b> of <b>Hydrochloric Acid</b> into all wells.

15. Cover plate with adhesive foil. Incubate **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). Remove the foil.



**Do not decant the supernatant thereafter!**

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

**Adrenaline**

**75 µL**

**Noradrenaline**

**15 µL**

**Dopamine**

**100 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 **75 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 **Adrenaline Antiserum** into all wells and cover plate with **Adhesive Foil**.
5. Incubate for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6. Remove the foil. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
7. Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
8. Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
9. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
10. Pipette **100 µL** of the **Substrate** into all wells and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). **Avoid exposure to direct sun light!**
11. Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
12. **Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

### 6.4 Noradrenaline ELISA

1. Pipette **25 µl** of the **Enzyme Solution** (refer to 6.1) into all wells of the **Noradrenaline Microtiter Strips**.
2. Pipette **15 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 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6. Remove the foil. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
7. Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
8. Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
9. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
10. Pipette **100 µL** of the **Substrate** into all wells and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). **Avoid exposure to direct sun light!**
11. Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
12. **Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

### 6.5 Dopamine ELISA

1. Pipette **25 µl** of the **Enzyme Solution** (refer to 6.1) into all wells of the **Dopamine 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 **Dopamine Antiserum** into all wells and cover plate with **Adhesive Foil**.
5. Incubate for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6. Remove the foil. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
7. Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
8. Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
9. Discard or aspirate the content of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.

	Pipette <b>100 µL</b> of the <b>Substrate</b> into all wells and incubate for <b>20-30 min</b> at <b>RT</b> (20-25°C) on a shaker (approx. 600 rpm). <b>Avoid exposure to direct sun light!</b>
<b>11.</b>	Add <b>100 µL</b> of the <b>Stop Solution</b> to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
<b>12.</b>	<b>Read</b> the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to <b>450 nm</b> and a reference wavelength between 620 nm and 650 nm.

## 7. Calculation of results

Standard	Concentration of the standards						A/B*
	A	B	C	D	E	F	
<b>Adrenaline (ng/mL)</b>	0	1	4	16	64	256	
<b>Adrenaline (nmol/L)</b>	0	5.46	21.8	87.4	349	1,398	
<b>Noradrenaline (ng/mL)</b>	0	4	16	64	256	1,024	
<b>Noradrenaline (nmol/L)</b>	0	23.6	94.6	378	1,513	6,052	
<b>Dopamine (ng/mL)</b>	0	10	40	160	640	2,560	2.5
<b>Dopamine (nmol/L)</b>	0	65.3	261	1,045	4,179	16,717	16.33
<b>Conversion:</b>	Adrenaline (ng/mL) x 5.46 = Adrenaline (nmol/L) Noradrenaline (ng/mL) x 5.91 = Noradrenaline (nmol/L) Dopamine (ng/mL) x 6.53 = Dopamine (nmol/L)						

⚠ *\*for the determination of dopamine in plasma the additional **Standard A/B** is mandatory!*

The calibration 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).

### Urine samples and controls:

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

### Plasma samples:

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

### 7.1 Quality control

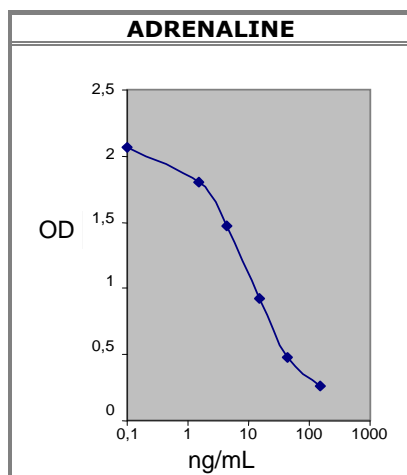
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC Report.

### 7.2 Calibration

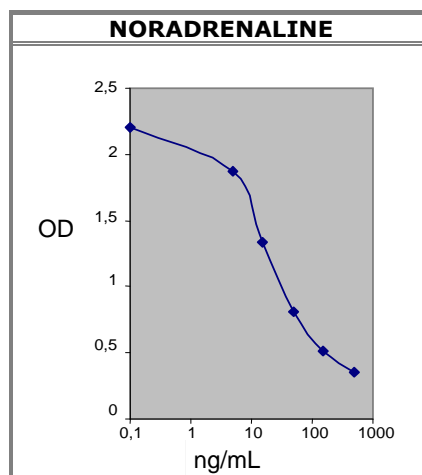
The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation 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*

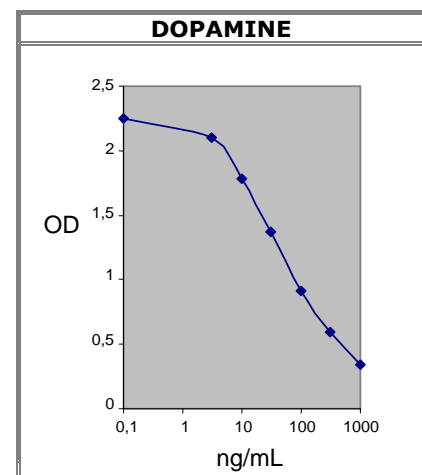
### 7.3 Typical calibration curves



⚠ *Example. Do not use for calculation!*



⚠ *Example. Do not use for calculation!*



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## 8. Assay characteristics

Expected Reference Values		Adrenaline	Noradrenaline	Dopamine
	Urine	< 20 µg/day (110 nmol/day)	< 90 µg/day (535 nmol/day)	< 600 µg/day (3,900 nmol/day)
	Plasma	< 100 pg/mL	< 600 pg/mL	< 100 pg/mL

Analytical Sensitivity (Limit of Detection)	Mean signal (Zero-Standard) - 2SD			
		Adrenaline	Noradrenaline	Dopamine
	Urine	0.33 ng/mL	1.33 ng/mL	0.83 ng/mL
Plasma	11 pg/mL	44 pg/mL	28 pg/mL	

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	

Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Noradrenaline	1	24.4 ± 3.9	16.1	Noradrenaline	1	39.8 ± 3.4	8.5
	2	92.7 ± 9.0	9.8		2	135 ± 20	15.0
Adrenaline	1	2.5 ± 0.4	15.0	Adrenaline	1	8.8 ± 1.1	13.2
	2	11.7 ± 0.8	6.9		2	34.2 ± 5.2	15.4
Dopamine	1	120 ± 11.5	9.5	Dopamine	1	148 ± 23.6	15.9
	2	1,074 ± 169	15.8		2	589 ± 107	18.2

Linearity			Range	Serial dilution up to	Range (%)
	Noradrenaline	Urine	20 - 339 ng/mL	1:16	85 - 123
		Plasma	318 - 2,436 pg/mL	1:8	84 - 123
	Adrenaline	Urine	4.6 - 81.4 ng/mL	1:16	86 - 124
		Plasma	92 - 545 pg/mL	1:8	81 - 121
	Dopamine	Urine	114 - 1,917 ng/mL	1:16	84 - 106
Plasma		1,000-8,000 pg/mL	1:8	74 - 131	

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Noradrenaline	Urine	109	83 - 115	
		Plasma	97	85 - 108	
	Adrenaline	Urine	107	84 - 119	
		Plasma	92	80 - 113	
	Dopamine	Urine	95	85 - 104	
Plasma		90	74 - 110		

Method Comparison versus HPLC*	Noradrenaline	HPLC = 1.27 ELISA - 0.04	r = 0.96; n = 30
	Adrenaline	HPLC = 1.17 ELISA - 0.06	r = 0.99; n = 30
	Dopamine	HPLC = 0.98 ELISA + 0.08	r = 0.95; n = 30

\* The concentrations were assessed using both the ELISA and the HPLC method (external QC samples from UK NEQAS). The correlation between ELISA and HPLC is excellent. This means, that the ELISA measure equally good when compared to the UK NEQAS HPLC data. Please take in mind, that the UK control values are the mean of about 40 different HPLC users, and contain always one pathological sample per sending.

## 9. **Advice on handling the test**

### 9.1 **Reliability of the test results**

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, 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.

### 9.2 **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 complaint form and return it completely filled in to the manufacturer.

### 9.3 **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.

### 9.4 **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 on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

### 9.5 **Interference**

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can 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.

### 9.6 **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.



**Actual literature or any other information about the test are available on the homepage or contact the manufacturer directly.**

## **Symbols:**

	Contains sufficient for <n> tests		Manufacturer		Storage temperature
	Catalogue number		Batch code		Expiry date
	For in-vitro diagnostic use only!		Content		Consult instructions for use
	For research use only!		Caution		