



User's Manual

Adrenaline - Noradrenaline - Dopamine ELISA Kit



DEIA05701



3x96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

For the quantitative determination of adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine in plasma and urine. **For Research Use Only. Not For Use In Diagnostic Procedures.**

General Description

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 the body's fight-or-flight response. The catecholamines and their metabolites indicate the adaptation of the body to acute and chronic stress.

Principles of Testing

Enzyme immunoassay for the quantitative determination of adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine in plasma and urine. For research use only. Not for use in diagnostic or therapeutic procedures.

Adrenaline (epinephrine), noradrenaline (norepinephrine), and dopamine are extracted using a cis-diol-specific affinity gel, then acylated and 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.

Reagents And Materials Provided

- 1. Adhesive Foil, Ready to use:** Adhesive foils in a resealable pouch. 3 × 4 foils
- 2. Wash Buffer Concentrate 50x:** Buffer with a non-ionic detergent at physiological pH. 3 × 20 mL/vial, light purple cap
- 3. Enzyme Conjugate, Ready to use:** Goat anti-rabbit immunoglobulin, conjugated with peroxidase. 3 × 12 mL/vial, red cap
- 4. Substrate, Ready to use:** Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide. 3 × 12 mL/vial, black cap
- 5. Stop Solution, Ready to use:** 0.25 M sulfuric acid. 3 × 12 mL/vial, light grey cap.
- 6. Adrenaline Microtiter Strips, Ready to use:** 1 × 96 well (12×8) antigen precoated microwell plate in a resealable blue pouch with desiccant

- 7. Noradrenaline Microtiter Strips, Ready to use:** 1 × 96 well (12×8) antigen precoated microwell plate in a resealable yellow pouch with desiccant
- 8. Dopamine Microtiter Strips, Ready to use:** 1 × 96 well (12×8) antigen precoated microwell plate in a resealable green pouch with desiccant
- 9. Adrenaline Antiserum, Ready to use:** Rabbit anti-adrenaline antibody, blue colored. 1 × 6 mL/vial, blue cap
- 10. Noradrenaline Antiserum, Ready to use:** Rabbit anti-noradrenaline antibody, yellow colored. 1 × 6 mL/vial, yellow cap
- 11. Dopamine Antiserum, Ready to use:** Rabbit anti-dopamine antibody, green colored. 1 × 6 mL/vial, dark green cap
- 12. Adjustment Buffer, Ready to use:** TRIS buffer. 2 × 4 mL/vial, green cap
- 13. Acylation Buffer, Ready to use:** Buffer with light alkaline pH for the acylation. 1 × 20 mL/vial, white cap
- 14. Acylation Reagent, Ready to use:** Acylation reagent in DMSO. 1 × 3 mL/vial, white cap
- 15. Assay Buffer, Ready to use:** 1M hydrochloric acid and a non-mercury preservative. 1 × 6 mL/vial, light grey cap
- 16. Coenzyme, Ready to use:** S-adenosyl-L-methionine. 1 × 4 mL/vial, purple cap
- 17. Enzyme, Lyophilized:** Catechol-O-methyltransferase. 6 vials, pink cap
- 18. Extraction Buffer, Ready to use:** Buffer containing carbonate. 1 × 6 mL/vial, brown cap
- 19. Extraction Plate, Ready to use:** 2 × 48 well plates coated with boronate affinity gel in a resealable pouch
- 20. Hydrochloric Acid, Ready to use:** 0.025 M Hydrochloric Acid, yellow colored. 1 × 20 mL/vial, dark green cap
- 21. STANDARD A:** 4mL
ADR 0ng/mL (0nmol/L), NAD 0ng/mL (0nmol/L), DOP 0ng/mL (0nmol/L).
- 22. STANDARD B:** 4mL
ADR 1ng/mL (5.5nmol/L), NAD 5ng/mL (30nmol/L), DOP 10ng/mL (65nmol/L).
- 23. STANDARD C:** 4mL
ADR 4ng/mL (22nmol/L), NAD 20ng/mL (118nmol/L), DOP 40ng/mL (261nmol/L).
- 24. STANDARD D:** 4mL
ADR 15ng/mL (82nmol/L), NAD 75ng/mL (443nmol/L), DOP 150ng/mL (980nmol/L).
- 25. STANDARD E:** 4mL
ADR 50ng/mL (273nmol/L), NAD 250ng/mL (1478nmol/L), DOP 500ng/mL (3265nmol/L).
- 26. STANDARD F:** 4mL
ADR 200ng/mL (1092nmol/L), NAD 1000ng/mL (5910nmol/L), DOP 2000ng/mL (13060nmol/L).
- 27. STANDARD A/B:** 4mL
ADR -ng/mL (-nmol/L), NAD -ng/mL (-nmol/L), DOP 4.5ng/mL (29nmol/L).

28. CONTROL 1: 4mL. Refer to QC-Report for expected value and acceptable range!

29. CONTROL 2: 4mL. Refer to QC-Report for expected value and acceptable range!

Conversion: Adrenaline (ng/mL) \times 5.46 = Adrenaline (nmol/L)

Noradrenaline (ng/mL) \times 5.91 = Noradrenaline (nmol/L)

Dopamine (ng/mL) \times 6.53 = Dopamine (nmol/L)

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

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

Materials Required But Not Supplied

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

Storage

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

Specimen Collection And Preparation

1. Plasma

Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant and centrifuged according to manufacturer' s instructions immediately after collection. In case of hemolytic, icteric, or lipemic samples see section **Interferences**.

Storage: up to 6 hours at 2 - 8°C, for longer period (up to 6 months) at -20°C. Repeated freezing and thawing should be avoided.

2. 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 months) at -20°C.

Repeated freezing and thawing should be avoided.

Avoid exposure to direct sunlight.

Reagent Preparation

Please read the **Assay Note** in the **Assay Procedure** section before operation.

1. Wash Buffer:

Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 mL. Storage: 2 months at 2 – 8 °C

2. Enzyme Solution:

Reconstitute the contents 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 fresh just prior to the assay (not longer than 10 to 15 minutes in advance). Discard after use!

3. Adrenaline Microtiter strips, Noradrenaline Microtiter Strips, and Dopamine 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.

4. Acylation Reagent:

The Acylation Reagent has a freezing point of 18.5°C. To ensure that the Acylation Reagent is liquid while in use, it must reach room temperature and form a homogeneous, crystal-free solution before use.

Assay Procedure

Assay Note:

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 before use to avoid any mix-up. The binding of the antiserum and the enzyme conjugate and the activity of the enzyme are temperature dependent; 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.

Sample preparation, extraction and acylation

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

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 175 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: Adrenaline 100 µL, Noradrenaline 20 µL, Dopamine (standards + urine) 25 µL, Dopamine (plasma) 50 µL

Adrenaline ELISA

1. Pipette 25 µL of the Enzyme Solution (refer to Reagent Preparation) 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 hours 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 times by adding 300 µL of 1× working Wash Buffer, discarding the contents 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 times by adding 300 µL of 1× working Wash Buffer, discarding the contents 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).

Noradrenaline ELISA

1. Pipette 25 µL of the Enzyme Solution (refer to Reagent Preparation) 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 hours 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 times by adding 300 µL of 1× working Wash Buffer, discarding the contents 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 times by adding 300 µL of 1× working Wash Buffer, discarding the contents 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).

Dopamine ELISA

1. Pipette 25 µL of the Enzyme Solution (refer to Reagent Preparation) into all wells of the Dopamine Microtiter Strips.
2. Pipette 25 µL of the extracted standards, controls, urine samples and 50 µL of the extracted plasma samples into the appropriate wells.
3. Add 25 µL of Hydrochloric Acid to the standards, controls, and urine samples.
4. Incubate for 30 min at RT (20 – 25°C) on a shaker (approx. 600 rpm).
5. Pipette 50 µL of the Dopamine Antiserum into all wells and cover plate with Adhesive Foil.
6. Incubate for 2 hours at RT (20 – 25°C) on a shaker (approx. 600 rpm).
7. Remove the foil. Discard or aspirate the content of the wells. Wash the plate 3 times by adding 300 µL of 1× working Wash Buffer, discarding the contents and blotting dry each time by tapping the inverted plate on absorbent material.
8. Pipette 100 µL of the Enzyme Conjugate into all wells.
9. Incubate for 30 min at RT (20 – 25°C) on a shaker (approx. 600 rpm).
10. Discard or aspirate the content of the wells. Wash the plate 3 times by adding 300 µL of 1× working Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
11. 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!

12. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
13. 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).

Calculation

Measuring range

Urine:

Adrenaline: 0.7 - 200 ng/mL

Noradrenaline: 2.5 – 1000 ng/mL

Dopamine: 4.8 – 2000 ng/mL

Plasma:

Adrenaline: 18 – 6667 pg/mL

Noradrenaline: 93 – 33333 pg/mL

Dopamine: 75 – 33333 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. 4-parameter, marquardt).

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 present.

Urine samples and controls

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

Calculate the 24 hour excretion for each urine sample: $\mu\text{g}/24\text{h} = \mu\text{g}/\text{L} \times \text{L}/24\text{h}$

Plasma samples

The read Adrenaline and Noradrenaline concentrations of the plasma samples must be divided by 30.

The read Dopamine concentrations of the plasma samples must be divided by 60.

Conversion

Adrenaline (ng/mL) \times 5.46 = Adrenaline (nmol/L)

Noradrenaline (ng/mL) \times 5.91 = Noradrenaline (nmol/L)

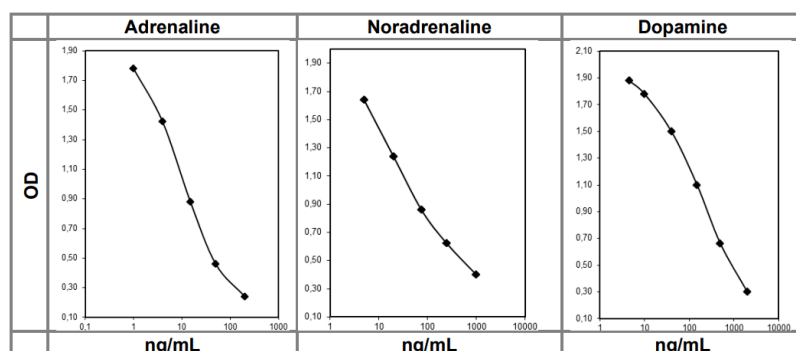
Dopamine (ng/mL) \times 6.53 = Dopamine (nmol/L)

Quality control

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

Typical Standard Curve

Examples, do not use for calculation!



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
Dopamine	1	82 ± 16.1	19.7	Dopamine	1	75 ± 22	29.8
	2	253 ± 41.1	16.3		2	353 ± 86	24.4
	3	714 ± 67	9.4		3	1187 ± 293	24.9

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
Dopamine	1	79.3 ± 18.8	23.7	Dopamine	1	238 ± 67.0	28.2
	2	222 ± 27.0	12.1		2	1072 ± 201	18.8
	3	630 ± 69.0	11.0		3	3449 ± 491	14.2

Detection Limit

Analytical Sensitivity			Adrenaline	Noradrenaline	Dopamine
	LOD	Urine (ng/mL)	0.9	1.7	2.5
		Plasma (pg/mL)	10	36	49
	LOQ	Urine (ng/mL)	0.7	2.5	4.8
		Plasma (pg/mL)	18	93	75

Specificity

Analytical Specificity (Cross-Reactivity)	Substance	Cross-Reactivity (%)		
		Adrenaline	Noradrenaline	Dopamine
	Derivatized Adrenaline	100	0.08	0.02
	Derivatized Noradrenaline	0.13	100	6.4
	Derivatized Dopamine	< 0.01	0.03	100
	Metanephrine	0.18	< 0.01	< 0.01
	Normetanephrine	< 0.01	0.16	0.01
	3-Methoxytyramine	< 0.01	< 0.01	0.49
	3-Methoxy-4-hydroxyphenylglycol	< 0.01	< 0.01	< 0.01
	Tyramine	< 0.01	< 0.01	0.18
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.01	< 0.01	< 0.01

Linearity

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
	Dopamine	Urine	1:512	83 - 126	104
		Plasma	1:512	85 - 132	106

Recovery

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 – 14251 pg/mL
	Dopamine	Urine	110	101-124	225 – 1306 ng/mL
		Plasma	89	84 - 92	57.4 – 16054 pg/mL

Interferences

Plasma:

Samples containing precipitates or fibrin strands or which are hemolyzed or lipemic may cause inaccurate results. Hemolytic samples (up to 4 mg/mL hemoglobin), icteric samples (up to 50 mg/dl bilirubin), and lipemic samples (up to 800 mg/dl triglycerides) have no influence on the assay results.

24-hour urine:

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

Drug interferences:

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

High-Dose-Hook effect:

No hook effect was observed in this assay.

Precautions

- (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 instructions provided with the kit are valid and must 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) must be followed.
- (3) To reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (4) All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
- (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 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 use should be marked accordingly to avoid any mix-up.
- (7) Duplicate determination of samples 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 and 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 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 the Safety Data Sheet (SDS).
- (17) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
- (18) Receipt of severely damaged test kits or components must be reported within 1 week of receipt. Severely damaged test components should not be used for a test run. They must be stored properly until the manufacturer determines if the materials may be used or must be discarded.

Limitations

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