Instructions for Use

Cortisol ELISA

Enzyme Immunoassay for the in-vitro-diagnostic quantitative determination of cortisol in human saliva and serum

REF **RE52611**

Σ **96**

EU: IVD CE U.S.: *For in-vitro diagnostic use only.*

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1. INTENDED USE

Enzyme immunoassay for the *in-vitro diagnostic* quantitative determination of free Cortisol in human saliva and of total Cortisol in diluted serum as an aid in the assessment of Cushing Syndrome and Addison's Disease.

2. SUMMARY AND EXPLANATION

Cortisol (hydrocortisone, compound F) is the main glucocorticoid in humans and is produced in the zona fasciculata of the adrenal cortex. 90 % of the circulating cortisol are bound to corticoid binding globulin (CBG, Transcortin), ca. 7 % are bound to albumin and only 1–3 % are unbound. Only the latter part represents the active form of cortisol.
The free cortisol is released in saliva and is excreted via the kidneys as a small part among the metabolites of cortisol. The level of free cortisol in blood regulates mainly its secretion in the adrenal cortex in a negative feedback mechanism via CRH (corticotropin releasing hormone) in the hypothalamic region and the ACTH in the pituitary gland, but it is also affected by different situations above all by stress.
In humans there is a physiological fluctuation of cortisol achieving the highest level in the morning and the lowest during midnight. This fluctuation of cortisol plasma level is reflected in saliva normally with a peak in the first 90 minutes after wake up.
The cortisol measurement is indicated in diseases with abnormal gluco-corticoid production e.g. Cushing Syndrome and Addison's Disease. Because of the diurnal fluctuation of cortisol levels it is necessary to take several samples for an individual cortisol profile or during dynamic tests like dexamethasone-suppression- or ACTH-stimulation-test. Therefore a salivary sample collection is an easy method without the stress of repeated venipunctures. The measurement of cortisol in saliva is advisable in patients with abnormal CBG levels such as women in pregnancy, people with hypothyroidism, nephrotic syndrome or marked adipositas and during the application of different drugs, including oral contraceptives.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

The patient should not eat, drink, chew gums or brush teeth for 30 min before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. Saliva flow can be stimulated by chewing on a piece of Parafilm®. It is recommended to freeze samples at –20°C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.

⚠ **Take care that the saliva samples are visually okay.**
(Reddish color indicating blood contamination)

Storage:	37°C	18-25°C	2-8°C	≤ -20°C (Aliquots)
Stability:	1 week	> 2 weeks	> 4 weeks	≥ 6 months

Serum, Plasma (EDTA)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	48 h	6 months	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	**MTP**	**Microtiter Plate** Break apart strips. Coated with anti-cortisol antibodies (rabbit).
1 x 13 mL	**ENZCONJ**	**Enzyme Conjugate** Yellow Colored. Ready to use. Contains: Cortisol (chromatographically purified), conjugated to HRP, stabilizers.
1 x 3.5 mL 6 x 1.0 mL	**CAL A-G**	**Standard A-G** 0; 0.03; 0.06; 0.20; 0.60; 1.50; 4.00 µg/dL 0; 0.3; 0.6; 2.0; 6.0; 15; 40 ng/mL 0; 0.83; 1.7; 5.5; 17; 41; 110 nmol/L Ready to use. Contains: Cortisol, Buffer, 0.1 % BSA, 0.1 % ProClin.
2 x 1.0 mL	**CONTROL 1+2**	**Control 1+2** Ready to use. Contains: Cortisol, low and high, Buffer, 0.1 % BSA, 0.1 % ProClin. Exact concentrations see vial labels or QC certficate.
1 x 12 mL	**TMB SUBS**	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 12 mL	**TMB STOP**	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
1 x 100 mL	**WASHBUF** **CONC**	**Wash Buffer** Concentrate (10x) Contains: phosphate buffer, Tween, stabilizers.
3 x	**FOIL**	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 5; 20; 50; 100; 1000 µL
2. A suitable sampling device should be used (can be ordered separately from IBL under REF RE69991)
3. Additional zero standard for serum dilution (can be ordered separately from IBL under REF KECO611)
4. Serum Controls (e.g. Lyphochek Immunoassay Plus Control, Biorad, Germany)
5. Orbital shaker (400-600 rpm)
6. Vortex mixer
7. 8-Channel Micropipettor with reagent reservoirs
8. Wash bottle, automated or semi-automated microtiter plate washing system
9. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
10. Bidistilled or deionised water
11. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
10 mL	WASHBUF	ad 100 mL	bidist. water	1:10	Mix vigorously.	2-8°C	4 weeks

10.2. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Saliva	no	-	-	-
Serum	generally	Standard A	1:50	e.g. 5 µL + 245 µL

Samples containing concentrations higher than the highest standard have to be diluted further up to 1:32 with Standard A and reassayed.

11. TEST PROCEDURE

1.	Pipette **50 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate.
2.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate with adhesive foil. Shake plate carefully.
3.	**Incubate 2 h** at **RT (18-25°C)** on an orbital shaker (400 – 600 rpm).
4.	Remove adhesive foil. Discard incubation solution. Wash plate **4 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
6.	**Incubate 30 min** at **RT (18-25°C)** on an orbital shaker (400 – 600 rpm).
7.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Shake briefly. Color changes from blue to yellow.
8.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

Due to the dilution of serum samples the serum values obtained have to be multiplied by the factor 50.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Saliva samples with remarkably elevated values should be reviewed for blood contamination.

Conversion:

Cortisol (ng/mL) x 2.76 = nmol/L

Cortisol (µg/dL) x 27.6 = nmol/L

Reportable range:

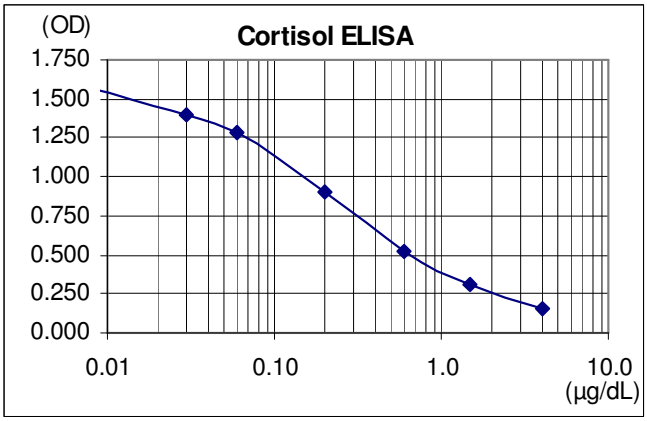
Saliva: 0.015 – 4 µg/dL Cortisol

Serum: 0.75 – 200 µg/dL Cortisol

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Cortisol (µg/dL)	OD_{Mean}	OD/OD_{max} (%)
A	0.00	1.699	100
B	0.03	1.401	82
C	0.06	1.278	75
D	0.20	0.905	53
E	0.60	0.524	31
F	1.50	0.304	18
G	4.00	0.155	9



Standards and Controls are calibrated by use of an isotope dilution-GCMS as reference method (Siekmann et al., J Clin Chem Clin Biochem 1982;20:883-892).

14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Apparently healthy subjects show the following values:

	n	µg/dL		nmol/L	
		AM	PM	AM	PM
Saliva	340	0.20 – 1.41	0.04 – 0.41	5.52 – 28.92	1.10 – 11.32
Serum	125	5 – 25	2 – 12	138 - 690	55.2 – 331.2

Time after awakening (h)	Cortisol (Saliva) Range (♂/♀; > 6 y; n = 110; 5% - 95% percentile)			
	Median (nmol/L)	Range (nmol/L)	Median (µg/dL)	Range (µg/dL)
0 – 1.5	18.9	5.1 - 40.2	0.685	0.185 – 1.457
1.5 – 3.0	11.8	3.6 - 28.4	0.428	0.130 – 1.029
3.0 – 6.0	6.7	2.1 - 15.7	0.243	0.076 – 0.569
6.0 – 9.0	5.5	1.8 - 12.1	0.199	0.065 – 0.438
9.0 – 15.0	3.3	0.9 - 9.2	0.120	0.033 – 0.333

(Westermann J, Demir A, Herbst V. Determination of Cortisol in Saliva and Serum by a Luminescence-Enhanced Enzyme Immunoassay. Clin Lab 2004;50:11-24)

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

Children levels have not yet been evaluated with this test.

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Note: Samples containing thimerosal should not be used in the assay.

The following blood components do not have a significant effect (+/- 20% of expected) on the test results up to the below stated concentrations:

	Saliva	
	Conc.	Cortisol (µg/dL)
Blood	0.25 %	0.16; 0.26; 1.09
NaN_3	0.25 %	0.18; 0.21; 0.33
	Serum	
	Conc.	**Cortisol (µg/dL)**
Hemoglobin	1.0 mg/mL	20.4; 15.8
Bilirubin	1.0 mg/mL	20.4; 15.8
Triglyceride	50 mg/mL	20.4; 15.8

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances tested < 0.01 %
	Prednisolone	30	
	11-Desoxy-Cortisol	7.0	
	Corticosterone	1.4	
	Cortisone	4.2	
	Prednisone	2.5	
	17α-OH-Progesterone	0.4	
	Desoxy-Corticosterone	0.9	
	6α-Methyl-17α-OH-Progesterone	0.04	
Analytical Sensitivity (Limit of Detection)	0.005 µg/dL	Mean signal (Zero-Standard) - 2SD	
Functional Sensitivity	0.030 µg/dL	Mean Conc. < 20 % CV	

Precision

	Saliva (n = 20)			Serum (n = 20)		
	Conc. (µg/dL)	SD (µg/dL)	CV (%)	Conc. (µg/dL)	SD (µg/dL)	CV (%)
Intra-Assay	0.27	0.019	7.3	1.8	0.174	9.9
	1.48	0.063	4.2	11.5	0.766	6.7
	2.34	0.073	3.1	26.2	1.554	5.9
Inter-Assay	0.54	0.047	8.8	1.5	0.305	20
	1.29	0.120	9.3	11.6	2.006	18
	2.35	0.151	6.4	20.3	2.712	13

Linearity

Saliva			Serum		
Dilution	Meas. (µg/dL)	Rec. (%)	Dilution	Meas. (µg/dL)	Rec. (%)
-	0.67	100	1:50	59.2	100
1:2	0.29	85	1:100	30.8	104
1:4	0.16	93	1:200	16.1	109
1:8	0.09	104	1:400	8.6	116
1:16	0.04	93	1:800	4.3	116
1:32	0.02	115	1:1600	1.7	91
-	0.33	100	1:50	59.3	100
1:2	0.17	104	1:100	29.4	99
1:4	0.08	99	1:200	16.4	111
1:8	0.04	98	1:400	7.5	101
1:16	0.02	107	1:800	4.3	117
1:32	0.01	117	1:50	1.7	94
-	1.65	100	1:100	70.9	100
1:2	0.83	100	1:200	35.7	101
1:4	0.41	102	1:400	18.5	104
1:8	0.21	113	1:800	10.2	115
1:16	0.10	107	1:1600	5.0	113
1:32	0.05	109	1:3200	2.2	99

Recovery

Saliva				Serum			
Conc. (µg/dL)	Added (µg/dL)	Meas. (µg/dL)	Rec. (%)	Conc. (µg/dL)	Added (µg/dL)	Meas. (µg/dL)	Rec. (%)
Saliva 1 (0.13)	-	0.13	100	Serum 1 (10.9)	-	10.9	100
	0.02	0.16	106		1.5	11.8	95
	0.06	0.17	87		3.1	13.8	99
	0.20	0.28	86		10.2	19.4	92
	0.60	0.61	83		30.6	38.4	92
	1.50	1.37	83		76.5	79.9	91
Saliva 2 (0.16)	-	0.16	100	Serum 2 (14.0)	-	14.0	100
	0.02	0.17	97		1.3	14.3	94
	0.06	0.20	92		2.6	14.9	90
	0.20	0.30	84		8.7	20.5	90
	0.60	0.66	87		26.0	33.8	85
	1.50	1.53	92		65.0	77.3	98
					173.4	191.2	102
Saliva 3 (0.15)	-	0.15	100	Serum 3 (19.5)	-	19.5	100
	0.02	0.16	91		1.5	21.4	102
	0.06	0.22	102		3.1	23.9	106
	0.20	0.33	94		10.2	28.6	96
	0.60	0.74	98		30.6	49.9	100
	1.50	1.54	88		76.5	93.0	97

Method Comparison

Saliva	IBL-ELISA = 1.09 x IBL-Luminescence IA + 0.01	r = 0.996; n = 82
Serum	IBL-ELISA = 1.06 x IBL-Luminescence IA - 1.44	r = 0.987; n = 60
	IBL-ELISA = 0.84 x GCMS + 0.36	r = 0.918; n = 33

17. PRODUCT LITERATURE REFERENCES

1 Determination of Cortisol in Saliva and Serum by a Luminescence-Enhanced Emzyme Immunoassay (IBL Jürgen Westermann, Anke Demir, Victor Herbst). Clin.Lab.50 11 - 24 (2004)

2 Kirschbaum C. et al. Impact of Gender. Menstrual Cycle Phase and Oral Contraceptives on the Activity of the Hypothalamus-Pituitary-Adrenal Axis. Psychosomatic Medicine 61 154 – 162 (1999)

3 Raff H.. J. L. Raff and J. W. FindLing. Late-Night Salivary Cortisol as a Screening Test for Cushing's Syndrome. J Clin Endocrinol Metab 83 2681 – 2686 (1998)

4 Morineau G. et al. Radioimmunoassay of cortisone in serum. urine and saliva to assess the status of the cortisol-cortisone shuttle. Clin Chem 43 1397 – 1407 (1997)

5 Tunn S. et al. Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. Clin Chem 38 1491 – 1494 (1992)

6 Shimada M. et al. Determination of salivary cortisol by ELISA and its application to the assessment of the circadian rhythm in children. Hom Res 44 213 – 217 (1995)

7 Thomopoulos P. et al. Long distance follow up of a patient with intermittent Cushing's disease by use of salivary cortisol measurements. In: Kirschbaum C. et al. (ed.) Assessment of hormones and drugs in saliva in biobehavioral research. 1st Edition. Hofgrebe & Huber. Seattle (1992) pp 89 – 92

8 Kahn J. P. et al. Applications of salivary cortisol determinations to psychiatric and stress research: stress responses in students during academic examinations. In: Kirschbaum C. et al. (ed.) Assessment of hormones and drugs in saliva in biobehavioral research. 1st Edition. Hofgrebe & Huber. Seattle (1992) pp 111 – 127

9 Laudet M. H. et al. Salivary Cortisol Measurement: A Practical Approach to Assess Pituitary-Adrenal Function. J Clin Endocrinol Metab 66 343 – 348 (1988)

10 Umeda T. et al. Use of Saliva for Monitoring Unbound Free Cortisol Levels in Serum. Clin Chem Acta 110 245 – 253 (1981)

11 Peters J. R. et al. Salivary Cortisol Assays for Assessing Pituitary-Adrenal Reserve. Clin Endocrinol 17 583 – 592 (1982)

12 Buchanan et al. Circadian regulation of cortisol after hippocampal damage in humans; BIOL PSYCHIATRY 2004; 56:651-656

13 Hellhammer et.al Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress; Stress 2004 Vol. 00 (0), pp. 1-8

14 Gaab et al. Psychological determinations of the cortisol stress response: the role of anticipatory cognitive appraisal; Psychoneuroendocrinology 2005 30, 599-610

15 Fries et al. Attenuation of the hypothalamic-pituitary-adrenal axis responsivity of the Trier social stress test by the benzodiazepine alprazolam; Psychoneuroendocrinology 2006 31, 1278-1288

16 Miller ete al. clinical depression and regulation of the inflammatory response during acute stress; Psychosomatic Medicine 2005 67:679-687

17 Kuhlmann et al. Effects of oral cortisol treatment in healthy young women on memory retrieval of negative and neutral words; Neurobiology of learning and memory 2005 83 158-162

18 Li et al. Life-time socio-economic position and cortisol patterns in mid-life; Psychoneuroendocrinology 2007 32, 824-833

19 Wolf et al. No morning cortisol response in patients with severe global amnesia; Psychoneuroendocrinology 2005 30, 101-105

20 Wessa et al. Altered cortisol awakening response in posttraumatic stres disorder; Psychoneuroendocrinology 2006 31, 209-215

21 Rohleder et al. Sex-specific adaption of endrocrine and inflammatory responses to repeated nauseogenic body rotation; Psychoneuroendocrinology 2006 31, 226-236

22 Rohleder et al. Stress on the dance floor: The cortisol stress response to social-evaluative threat in competitive ballroom dancers; PSPB Vol. 33 No. 1, January 2007 69-84

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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