DATA SHEET

HMGB1 ELISA

Enzyme immunoassay for the quantitative determination of HMGB1 in human serum and plasma. Further the test can be used for research of serum, plasma and CSF from bovine, pig, rabbit, mouse and rats, as well as cell culture medium and BALF.

REF **ST51011**

Σ **12x8**

2-8 °C

EU: IVD CE U.S.: *For research use only.*
Not for use in diagnostic procedures.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

1. INTENDED USE

Enzyme immunoassay for the quantitative determination of HMGB1 in human serum and plasma.
Further the test can be used for research of serum, plasma and CSF from bovine, pig, rabbit, mouse and rats, as well as cell culture medium and BALF.

2. SUMMARY AND EXPLANATION

HMGB1 is an approximately 30 kDa protein that is the major component of the non-histone nuclear protein group. HMGB1 (High Mobility Group Box1 Protein) is a transcriptional regulator and is normally present in the nucleus and cytoplasm of mammalian cells.
HMGB1 plays an important role in disease pathogenesis, such as malignant diseases. In case of a Tumor the oxygen supply of cells could be disconnected. This leads to cell death (necrosis) and release of HMGB1, which causes the formation of angiogenetic blood vessel in the adjacent tumor environment towards the tumor. These vessels assure the tumor survival and further growth.
In vitro studies have shown that via quantitative determination of HMGB1 concentrations it is possible to differentiate between pathological cell death (necrosis) and physiological cell death (apoptosis). In necrosis much higher HMGB1 values are observed.
HMGB1 also plays an important role in the late phase of septic shock. It was shown that the inhibition of HMGB1 in animal experiments even in the late phase of septic shock significantly enhanced the survival rate of rodents. HMGB1 acts upon binding to RAGE (“receptor for advanced glucated end products”) and has a similar pro-inflammatory effect as the cytokines TNF- α and IL-6. Elevated values can also be found in the serum of septic patients.
HMGB1 concentration in blood is also increased in many other diseases including rheumatoid arthritis (RA) acute lung injury (ALI) and disseminated intravascular coagulation (DIC).
The HMGB1 ELISA was developed by Prof. Dr. Ikuro Maruyama and Shino-Test Corporation (Japan). This product is manufactured by IBL under the licence of Shino-Test Corporation.

3. TEST PRINCIPLE

HMGB1 ELISA is a Sandwich-enzyme immunoassay for the quantitative determination of HMGB1 in serum and plasma. The wells of the microtiter strips are coated with purified anti-HMGB1 antibody. HMGB1 in the sample binds specifically to the immobilized antibody and is recognized by a second enzyme marked antibody. After substrate reaction the HMGB1 concentration is determined by the colour intensity.

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. Avoid contact with Stop solution. It may cause skin irritations and burns.

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

Serum, Plasma (EDTA)

The usual precautions for venipuncture should be observed. Collect serum/plasma immediately from blood collection device after centrifugation for 15 minutes at over 1.000 g. HMGB1 level may increase due to cytolysis, if the centrifugation is not sufficient or the serum/plasma is in contact with blood cells for a considerable period of time.

Storage:	2-8°C (Aliquots)	- 30°C (Aliquots)	- 80 °C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	24 h	6 mon	12 mon	

HMGB1 can also be measured in cell culture supernatant, cerebrospinal fluid (CSF) and bronchoalveolar fluid (BALF). In the case of cell culture supernatant in fetal bovine serum (FBS), bovine HMGB1 is measured. Therefore a negative control should be also tested.

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-HMGB1 (polyclonal).
1 x	ENZCONJ LYO	**Enzyme Conjugate** (lyophilized) Contains: HMGB1,2 conjugated to peroxidase. For dilution see chapter 10.1.
1 x	CAL LYO	**Standard** (lyophilized) Contains: HMGB1 (pig) For dilution see chapter 10.1.
1 x	CONTROL+ LYO	**Positive Control** (lyophilized) Contains: HMGB1 (pig) For dilution see chapter 10.1.
1 x 20 mL	DILBUF	**Diluent Buffer** Ready to use. Contains: Buffer, 0.01 % NaN_3.
1 x 12 mL	ENZCONJDIL	**Enzyme Conjugate Diluent** Ready to use. Contains: Buffer.
2 x 100 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (5 x) Contains: phosphate buffer, <0.5 %Tween 20.
1 x 6 mL	COLREA A	**Colour reagent A** Ready to use. Contains: TMB.
1 x 6 mL	COLREA B	**Colour reagent B** Ready to use. Contains: Buffer with 0.005 M hydrogen peroxide.
1 x 12 mL	COLOUR STOP	**Colour Stop Solution** Ready to use. Contains: 0.35 M H_2SO_4.
2 x	FOIL	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 10; 20; 100; 100-1000 µL
2. Vortex mixer
3. Orbital shaker (500 rpm)
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer
9. Polystyrene (PS) or polypropylene (PP) tubes for standard and sample dilution
10. +37 °C and +25 °C Incubator

9. PROCEDURE NOTES

11. This test can be performed with two different measurement ranges. Two aliquots of the patient specimen should be stored in case the sample is out of the concentration range of the standard curve used.

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.

12. There is no influence in test results by crystal formation in the wells. There is no need to wash the wells before starting the test!

13. Each test run needs a standard curve.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
	CAL LYO	see Label	DILBUF	End conc.: 320 ng/mL	Aliquots (PS or PP tubes)	< -30 °C	1 mon
	CONTROL+ LYO	see Label	DILBUF		Aliquots (PS or PP tubes)	< -30 °C	1 mon
12 mL	**ENZCONJ LYO**	12 mL	ENZCONJDIL		Aliquots (PS or PP tubes). Store protected from light. Avoid repeated freeze-thaw cycles.	< -30 °C or 18-25°C	1 mon 5 h
Examples for 32 wells							
50 mL	**WASHBUF CONC**	200 mL	bidist. water	1:5	Aliquots	-	-
1.8 mL	**COLREA A**	1.8 mL	COLREA B	1:2 (1+1)	Prepare immediately before needed.	18-25°C	3 h

⚠
1. Add specified amount to the vial.
2. Shake gently by hand for a few second. Avoid allowing the solution to contact the rubber stopper as the material may be adsorbed to the rubber.
3. Leave at RT for at least 10 min to ensure complete reconstitution.
4. Swirl gently before use. Avoid contact of the solution and the rubber stopper.

10.2. Preparation of standard curve

10.2.1. Normal range standard curve

Concentration (ng/mL)	Dilution method	Standard
80	Add 100 µL of standard solution (320 ng/mL) to 300 µL of Diluent buffer and mix.	7
40	Add 100 µL of **Standard 7** to 100 µL of Diluent buffer and mix.	6
20	Add 100 µL of **Standard 6** to 100 µL of Diluent buffer and mix.	5
10	Add 100 µL of **Standard 5** to 100 µL of Diluent buffer and mix.	4
5	Add 100 µL of **Standard 4** to 100 µL of Diluent buffer and mix.	3
2.5	Add 100 µL of **Standard 3** to 100 µL of Diluent buffer and mix.	2
0	Diluent buffer only.	1

10.2.2. High sensitive range standard curve

Concentration (ng/mL)	Dilution method	Standard
10	Add 20 µL of standard solution (320 ng/mL) to 620 µL Diluent buffer and mix.	7
5	Add 150 µL of **Standard 7** to 150 µL of Diluent buffer and mix.	6
2.5	Add 150 µL of **Standard 6** to 150 µL of Diluent buffer and mix.	5
1.25	Add 150 µL of **Standard 5** to 150 µL of Diluent buffer and mix.	4
0.625	Add 150 µL of **Standard 4** to 150 µL of Diluent buffer and mix.	3
0.313	Add 150 µL of **Standard 3** to 150 µL of Diluent buffer and mix.	2
0	Diluent buffer only.	1

10.3. Dilution of Samples

Samples containing concentrations higher than the highest standard have to be diluted with Diluent buffer in PS or PP tubes.

11. TEST PROCEDURE

	Normal range	**High sensitive range**
1.	**Pipette 100 µL** of **Diluent buffer** into the respective wells of the microtiter plate.	**Pipette 50 µL** of **Diluent buffer** into the respective wells of the microtiter plate.
2.	**Pipette 10 µL** of **Diluent buffer** into the **blank-well** of the microtiter plate.	**Pipette 50 µL** of **Diluent buffer** into the **blank-well** of the microtiter plate.
3.	**Pipette 10 µL** of **standard, positive control** and of each **serum** and **plasma sample** into the respective wells of the Microtiter Plate. Shake briefly 30 seconds.	**Pipette 50 µL** of **standard**, **positive control** and of each **serum** and **plasma sample** into the respective wells of the Microtiter Plate. **Shake briefly 30 seconds.**
4.	**Cover** plate with adhesive foil. **Incubate 20 – 24 hours** at **+37 °C.**	**Cover** plate with adhesive foil. **Incubate 20 – 24 hours** at **+37 °C.**
5.	Remove adhesive foil. Discard incubation solution. Wash plate **5 x** with **400 µL** diluted **Wash buffer**. Remove excess solution by tapping the inverted plate on a paper towel.	Remove adhesive foil. Discard incubation solution. Wash plate **5 x** with **400 µL** diluted **Wash buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
6.	Pipette **100 µL Enzyme conjugate** into each well.	Pipette **100 µL Enzyme conjugate** into each well.
7.	Cover plate with adhesive foil. **Incubate 2 hours** at **+25 °C.**	Cover plate with adhesive foil. **Incubate 2 hours** at **+25 °C.**
8.	Remove adhesive foil. Discard incubation solution. Wash plate **5 x** with **400 µL** diluted **Wash buffer**. Remove excess solution by tapping the inverted plate on a paper towel.	Remove adhesive foil. Discard incubation solution. Wash plate **5 x** with **400 µL** diluted **Wash buffer**. Remove excess solution by tapping the inverted plate on a paper towel.

9.	For adding of Colour solution and Stop Solution use, if available, an 8-channel micropipettor. Pipetting should be carried out in the same time intervals for Colour solution and Stop Solution. Use positive displacement and avoid formation of air bubbles.	For adding of Colour solution and Stop Solution use, if available, an 8-channel micropipettor. Pipetting should be carried out in the same time intervals for Colour solution and Stop Solution. Use positive displacement and avoid formation of air bubbles..
10.	Pipette **100 µL Colour solution** into each well.	Pipette **100 µL Colour solution** into each well.
11.	**Incubate 30 min** at **RT (18-25°C).**	**Incubate 30 min** at **RT (18-25°C).**
12.	Stop the colour reaction by adding **100 µL of Stop solution** into each well. Briefly mix contents by gently shaking the plate.	Stop the colour reaction by adding **100 µL of Stop solution** into each well. Briefly mix contents by gently shaking the plate.
13.	Clean the back of the wells. Be careful not to scratch the wells as this may interfere with measurements.	Clean the back of the wells. Be careful not to scratch the wells as this may interfere with measurements.
14.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 min**.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 min**.

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 vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

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 Logisitcs 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.

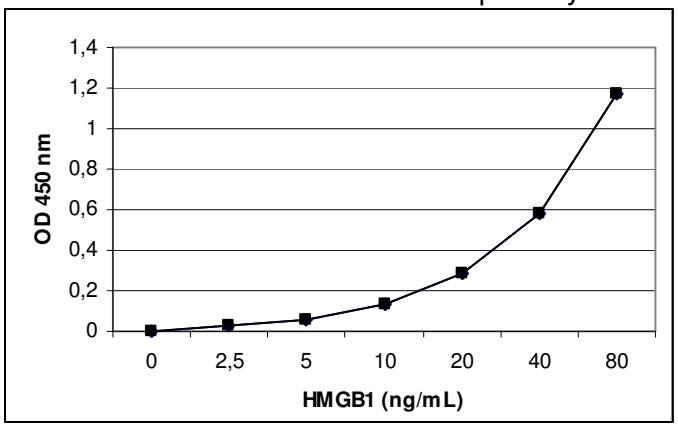
Samples showing concentrations above the highest standard have to be diluted and reassayed.

If samples have been diluted, the concentration read from the standard curve must be multiplied by dilution factor.

Typical Calibration Curve in the normal range

(example. Do not use for calculation!!)

Standard	HMGB1 (ng/mL)	OD
1	0	0.000
2	2.5	0.027
3	5	0.061
4	10	0.137
5	20	0.281
6	40	0.585
7	80	1.172

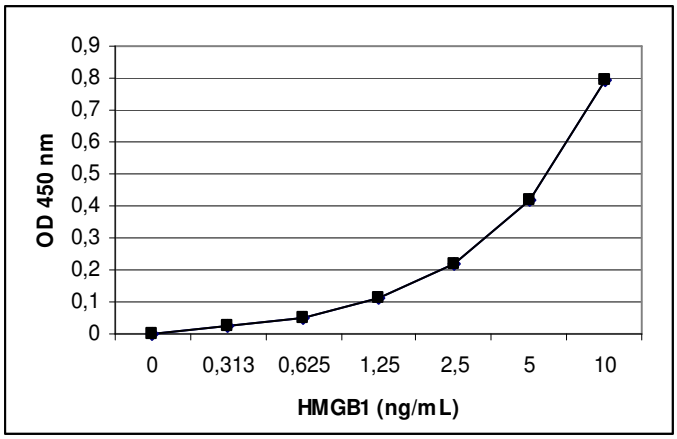


Samples with calculated concentrations below the lowest standard should be reassayed according to the high sensitive method.

Typical Calibration Curve in the high sensitive range

(example: Do not use for calculation!!)

Standard	HMGB1 (ng/mL)	OD
1	0	0.000
2	0.313	0.027
3	0.625	0.052
4	1.25	0.114
5	2.5	0.218
6	5	0.418
7	10	0.793



14. INTERPRETATION OF RESULTS

HMGB1 (Serum)	Interpretation
< 1.4 ng/mL	normal
> 1.4 ng/mL	elevated

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.

15. EXPECTED VALUES

Sera from 142 apparently healthy subjects were examined. Normal values were measured between 0 and 1.8 ng/mL. The upper limit of the reference range was found at 1.4 ng/mL (97.5 % percentile).

N	mean (ng/mL)	SD (ng/mL)	95 % percentile (ng/mL)	97.5 % percentile (ng/mL)	Cut-off	Values > 1.4 ng/mL (%)
142	0.39	0.42	1.2	1.4	1.4	2.5

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

16. LIMITATIONS OF THE PROCEDURE

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

For cross-reactivities, see PERFORMANCE.

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

RF	500 IU/mL
Bilirubin	50 mg/dL
Chyle	3000 FTU

Hemolytic samples should not be used. HMGB1 released from erythrocytes gives false high value by this assay. Also hemoglobin is known to positively interfere with the assay.

Insufficiently centrifuged samples or samples contaminated with erythrocytes could lead to false high results. See PRE-TEST SETUP INSTRUCTIONS.

17. PERFORMANCE

Analytical Specificity (Cross-reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances are not tested.		
	HMGB2	< 2.0			
Analytical Sensitivity (Limit of Detection)	1.0 ng/mL (normal range) 0.2 ng/mL (high sensitive range)	Mean signal (Zero-Standard) + 2.6 SD			
Functional Sensitivity (Limit of Quantification)	0.2 ng/mL (normal range) 0.1 ng/mL (high sensitive range)	Lowest HMGB1 concentration with CV ≤ 20%.			
Precision		Range (ng/mL)	CV (%)		
Intra-Assay	5 Samples (high sensitive range)	0.2 - 10	10.2 – 3.2		
	6 Samples (normal range)	2.3 - 80	13.7 – 5.5		
Inter-Assay	5 Samples (high sensitive range)	0.37 – 10	10.7 – 1.3		
	4 Samples (normal range)	9.7 – 77	13.7 – 7.6		
Linearity		Range (ng/mL)	Serial dilution up to	Range (%)	
	Serum (high sensitive range)	1.3 – 10.5	1:8	90.8 – 115.6	
	Serum (normal range)	2.1 – 103.2	1:16	93.1 – 109.9	
Recovery		Mean (%)	Range (%)		
	Serum (high sensitive range)	93.4	86.6 – 100.2	% Recovery after spiking	
	Serum (normal range)	99.6	94.7 – 104.6		

18. PRODUCT LITERATURE REFERENCES

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3. **Devaraj S, Dasu MR, Park SH, Jialal I.** *Increased levels of ligands of Toll-like receptors 2 and 4 in type 1 diabetes.* Diabetologia. 2009 August; **52(8)**: 1665–1668
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10. **Yamada S, Inoue K, Yakabe K, Imaizumi H, Maruyama I.** *High mobility group protein 1 (HMGB1) quantified by ELISA with a monoclonal antibody that does not cross-react with HMGB2.* Clin Chem. 2003 Sep;**49(9)**:1535-7

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.

LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer