Instructions for Use

Rheumatoid Factor (RF) IgA/IgG/IgM ELISA

Enzyme immunoassay for the separate quantitative detection of IgG, IgA and IgM rheumatoid factors (RF) in human serum

REF **RE70341**

Σ **96**

2–8 °C

EU: IVD CE

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

I B L I N T E R N A T I O N A L G M B H
Flughafenstrasse 52a
D-22335 Hamburg, Germany
Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11
IBL@IBL-International.com
www.IBL-International.com

Instruction manual

Contents

1. Intended Use

The Rf-AGM is a solid phase enzyme immunoassay with highly purified Fc fragment of human immunoglobuline (IgG) for the separate quantitative and qualitative detection of IgG, IgM and IgA rheumatoid factors (RF) in human serum.
The assay is an aid in the diagnosis of rheumatoid arthritis (RA).

2. Clinical Application and Principle of the Assay

Rheumatoid factors (RF), first described in 1940 as antibodies reacting with gamma globulins, are autoantibodies directed against the C-terminal part of the constant region of the IgG heavy chain, the IgG Fc.
Although named after the disease they were initially associated with, RFs are found both in the healthy population and several diseases. The diseases commonly associated with high RF concentrations are rheumatoid arthritis (RA; 50-90%) and Sjögren`s syndrome (75-95%). They are also found in systemic lupus erythematosus (SLE; 15-35%), systemic sclerosis (20-30%) polymyositis / dermatomyosisits (5-10%), cryoglobulinemia (40-100%) and mixed connective tissue diseases (MCTD; 50-60%).
Although the presence of IgM RF in the serum has been regarded as the most important serological indicator for RA, thus included in the ACR list of criteria for the diagnosis of this disease, RF of IgG and IgA subclass are important for diagnosis as well.
Determination of these isotypes provides additional information with regard to diagnosis, differential diagnosis and follow-up of RA in comparison to conventional techniques such as latex agglutination test and nephelometry. Whilst RF of subclass IgM are most sensitive for diagnosis of RA, thus most suitable for screening, RF of subclass IgG are most specific for RA and like subclass IgA correlate with clinical parameters and disease activity. The presence of all three subclasses together is 100 % specific for RA.
RF in SLE are associated with sicca syndrome, hypergammaglobulinemia, high titer of anti-nuclear antibodies, anemia and usually SS-A and SS-B antibodies appearance. All three subclasses are found in SLE. Especially the subclass IgA defines a subgroup of SLE patients characterized by distinct autoimmune phenomena and high disease activity in the absence of nephritis.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient‘s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

3. Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide < 0.1% and thimerosal 0,01% (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide < 0.1% and thimerosal 0,01% (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide < 0,1% (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide < 0,1% (preservative)

Cut-off Control 1 vial, 1.5 ml (capped blue: yellow solution)
Containing: Human serum (diluted), sodium Azide < 0,1% (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration: yellow solutions)
Containing: Human serum (diluted), sodium azide < 0,1% (preservative)

Conjugates 1 vial 15 ml IgA (capped red: red solution)
1 vial 15 ml IgG (capped blue: blue solution)
1 vial 15 ml IgM (capped green: green solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidaseand thimerosal 0,01% (preservative)

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H_2O_2

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.
Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C/39°F, at least. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.***

5. Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety :

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.
Do not smoke, eat or drink when manipulating the kit.
Do not pipette by mouth.
All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-26°C/64-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/ 98,6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples

Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B

NOTE: If IgG, IgA and IgM are determined in parallel, calibrators, controls and samples have to be done for each subclass separately.

- Pipette 100 µl of each patient‘s diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C/64-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F), in the dark.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Quantitative and Qualitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **U/ml .**

Normal Range	Positive Results
≤ 15 U/ml	**> 15 U/ml**

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgA/IgG/IgM	*OD 450/620 nm*	*CV % (Variation)*
0 U/ml	0.035	2.3
3 U/ml	0.138	2.6
10 U/ml	0.342	3.2
30 U/ml	0.632	3.2
100 U/ml	1.216	0.5
300 U/ml	2.178	0.1

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.872/0.922	0.897	54.7
P 02	1.159/1.188	1.174	86.3

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations. ***Do not use this example for interpreting patients results!***

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For **qualitative interpretation** read the optical density of the cut-off control and the patient samples. Compare patient´s OD with the OD of the cut-off control. All samples which are higher than cut-off are considered positive.

Negative:	**OD patient < OD $_{\text{cut-off}}$**
Positive:	**OD patient > OD $_{\text{cut-off}}$**

9. Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	60 minutes at room temperature (20-26°C/64-78.8°F)
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F use original vials, only
Number of determinations:	96 tests

10. Performance Data

10.1 Analytical sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity and sensitivity

The microplate is coated with **Fc fragment of human immunoglobuline (IgG).** No crossreactivities to other autoantigens have been found. Rheumatoid factors are detected in 70-90% of patients with rheumatoid arthritis (RA).

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	51.1	53.4	95.7
	1 / 200	25.2	26.7	94.4
	1 / 400	12.4	13.4	92.5
	1 / 800	6.3	6.7	94.0
2	1 / 100	135.1	138.0	97.9
	1 / 200	74.0	69.0	107.2
	1 / 400	32.1	34.5	93.0
	1 / 800	16.1	17.3	93.0

10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-Assay			Inter-Assay		
Sample No.	**Mean (U/ml)**	**CV (%)**	**Sample No.**	**Mean (U/ml)**	**CV (%)**
1	15.2	0.4	1	18.3	1.0
2	43.4	4.5	2	52.1	4.6
3	288.8	8.9	3	322.7	8.2

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml) for IgG and IgA rheumatoid factors. For IgM rheumatoid factor, the assay is calibrated against the international WHO standard and results are given in IU/ml.

11. Literature

1. **Peter JB, Shoenfeld Y (1996).**
   Autoantibodies.
   Elsevier Sciences B.V., Amsterdam.

2. **Witte T, Hartung K, Sachse C, Matthias T, Fricke M, Kalden JR, Lakomek HJ, Peter HH, Schmidt RE (2000).**
   Rheumatoid factors in systemic lupus erythematosus: Association with clinical and laboratory parameters. SLE study group.
   Rheumatol Int 19: 107-111.

ANNEX A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:
For **quantitative interpretation** use calibrators to establisch a standart curve
For **qualitative interpretation** use cut-off control

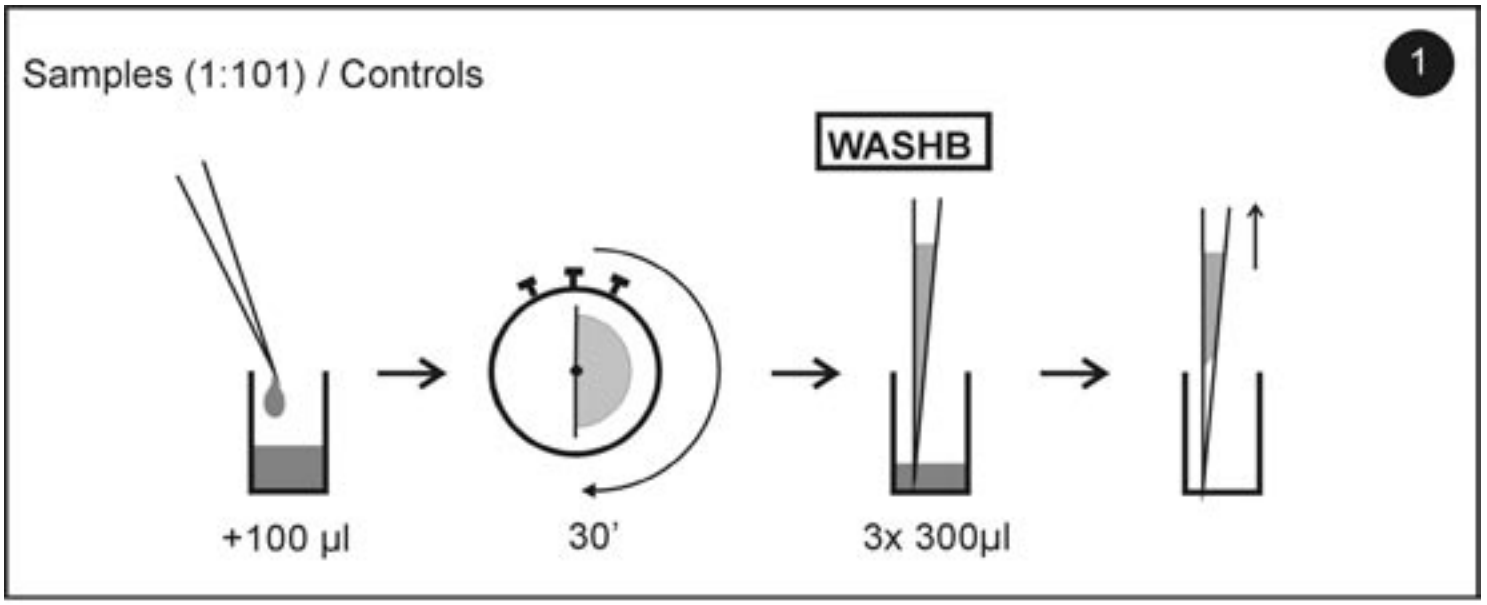
ANHANG A: Pipettierschema

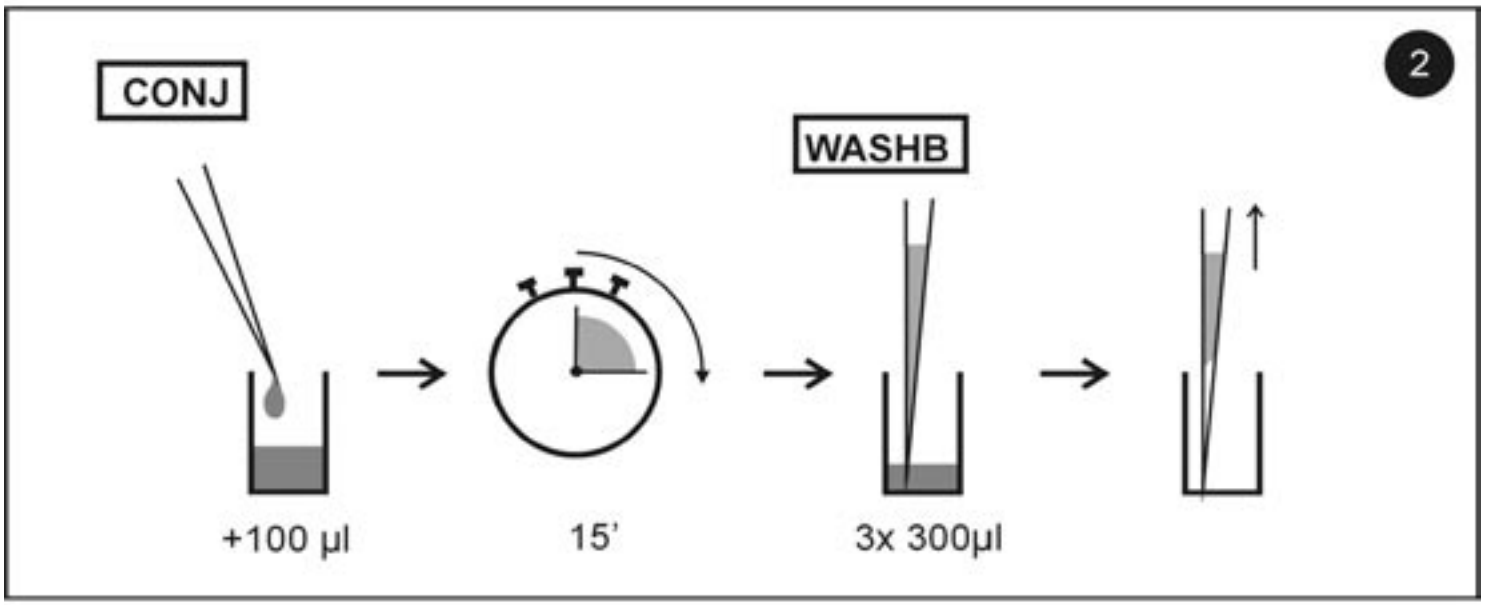
Wir empfehlen, die Kalibratoren, Kontrollen und Proben wie folgt zu pipettieren:
Zur **quantitativen Auswertung** verwenden Sie die Kalibratoren zur Erstellung einer Standardkurve.
Zur **qualitativen Auswertung** verwenden Sie die Cut-off Kontrolle.

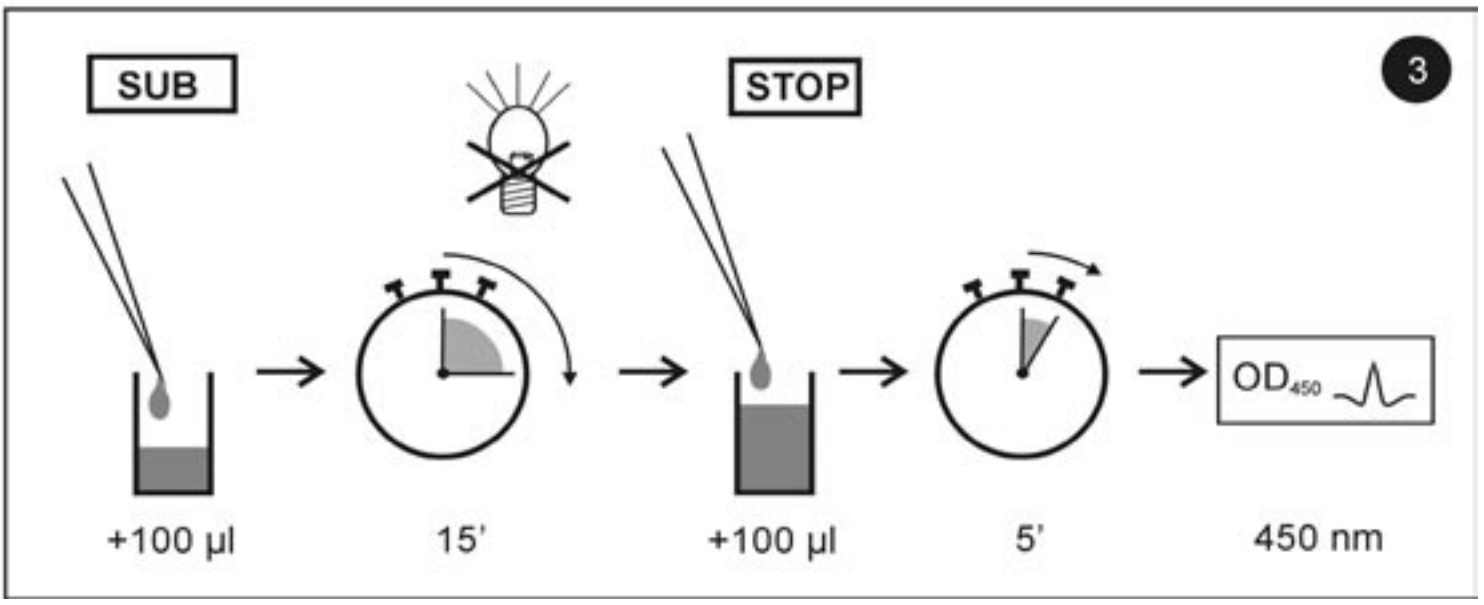
	for **quantitative interpretation** use calibrators to establish a standard curve						for **qualitative interpretation** use cut-off control					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	CalA	CalE	P1				NC	P2				
B	CalA	CalE	P1				NC	P2				
C	CalB	CalF	P2				CC	P3				
D	CalB	CalF	P2				CC	P3				
E	CalC	PC	P3				PC	...				
F	CalC	PC	P3				PC	...				
G	CalD	NC	...				P1	...				
H	CalD	NC	...				P1	...				

CalA: calibrator A, CalB: calibrator B, CalC: calibrator C, CalD: calibrator D, CalE: calibrator E, CalF: calibrator F
PC: positive control
NC: negative control
CC: Cut-off control
P1: patient 1
P2: patient 2
P3: patient 3

Annex B: Test Procedure/ Testablauf/







Assay/Test: _____ Incubation / Inkub. : 1._____min Date/ Datum:_____

Temperature/Temperatur:_____ °F _____ °C 2._____min

Signature/Unterschrift:._____

Name:_____ 3._____min

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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.

IBL AFFILIATES WORLDWIDE

IBL International GmbH Flughafenstr. 52A, 22335 Hamburg, Germany	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International B.V. Zutphenseweg 55, 7418 AH Deventer, The Netherlands	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International Corp. 194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada	Tel.: E-MAIL: WEB:	+1 (416) 645 -1703 Fax: -1704 Sales@IBL-International.com http://www.IBL-International.com

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