

# Autoantibodies against Islet Cells (ICA) ELISA

Enzyme Immunoassay for the qualitative detection of circulating autoantibodies against islet cell antigens.



**NM59081**



**12x8**

For illustrative purposes only.

To perform the assay the instructions for use provided with the kit have to be used.

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# 1. Introduction

Insulin-dependent diabetes mellitus (IDDM) or Type I Diabetes is a debilitating chronic disease that impairs production and secretion of the key hormone insulin and alters blood sugar metabolism. Insulin is synthesised and secreted by pancreatic islet cells or islets of Langerhans. The disruption of insulin synthesis is caused by immunological destruction of the islet cells by autoantibodies in IDDM patients. Such abnormalities (autoimmunity) may be genetically inherited and/or triggered by exposure to toxic chemicals, viral infections and various forms of stress.

IDDM has a characteristic asymptomatic prediabetic phase that may last up to several years. During this period, the affected individuals exhibit the diminishing early-phase release of insulin in response to an intravenous/oral glucose challenge. In the majority of cases, these individuals carry circulating islet cell autoantibodies (ICA) and/or insulin autoantibodies (IAA). ICA can be detected as early as eight years prior to the clinical onset of IDDM and thus may serve as an early indicator of the disease or of predisposition to it. Individuals who are ICA-positive may show a progressive loss of the islet cell function as indicated by disruption of the early-phase insulin release. When this early phase insulin release completely stops, clinically overt IDDM develops.

ICA are present in 70 % of patients with recent onset of IDDM compared with 0.1 - 0.5 % of the control non-diabetic population. ICA are also detected in first degree relatives of IDDM patients. These individuals comprise the segment of human population who are at a high risk of developing IDDM. Several studies reported that the ICA-positive first degree relatives of IDDM patients subsequently developed diabetes. Other studies also suggested that the presence of serum ICA and IAA is an indicator or the enhanced likelihood to develop IDDM. Therefore, serological detection of ICA may be a powerful tool for early diagnosis of IDDM. The significance of these autoantibodies as markers of IDDM is also illustrated by their presence in nondiabetic individuals who ultimately develop IDDM. Riley, et. al. recently reported that determination of ICA in Type 2 Diabetes patients could identify IDDM prior to the onset of clinical symptoms and predict the need for insulin therapy. Thus, those patients who are initially diagnosed with Type 2 Diabetes and carry serum ICA may deteriorate to insulin dependence.

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Early detection of circulating ICA is important to identify the individuals in the general population, the siblings and families of IDDM patients who are at a higher risk of developing this disease because of their genetic predisposition to diabetes. At a recent international workshop on ICA, the imminent need for an ELISA test for the determination of islet cell autoimmunity was emphasized.

Currently, serum ICA are determined by indirect immunofluorescence and histochemical methods employing frozen unfixed human/primate or rat pancreatic sections as substrates. Despite various attempts to improve and modify this procedure since its original description in 1974, the indirect immunofluorescence/histochemical technique suffers from inherent methodological problems. Standardisation of the technique has proven to be very difficult. The reliability of this "frozen-section" technique is limited by factors such as the variation from one pancreas to another, the inevitable need for unfixed pancreatic tissue and infrequent availability of the suitable tissue.

This ICA test is a qualitative ELISA test for in vitro detection of circulating IgG antibodies against pancreatic islet cell antigens.

## **2. Principle of the Test**

The highly purified mixture of pancreatic antigens is immobilized onto microwells. The positive control, negative control, and one dilution of patient serum are added to the appropriate microwells. Antibodies in the serum sample are allowed to react at room temperature with the antigen molecules on microwells. After washing off excess/unbound serum materials, the bound antibodies are determined by an enzyme labelled goat antibody specific to human IgG. After thorough washing, a substrate is added and the colour generated is measured spectrophotometrically. The intensity of the colour is directly proportional to the concentration of ICA in the sample. An ICA-positive control serves as an internal quality control and ensures valid results. The negative control is to indicate if washing is complete.

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### **3. Precautions**

- The assay calibrators and controls are of human origin and have been tested and confirmed negative for HIV, HBsAg and HCV by FDA approved procedures. All standards, however, should be treated as potential biohazards in use and for disposal.
- The assay reagents contain sodium azide or thimerosal which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
- This kit is for in vitro diagnostic use only.
- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
- Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

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## 4. Kit Components

### 4.1 Contents of the Kit

This test kit contains reagents and materials for 96 microwells.

<b>4.1.1 Microwell Strips (with the holder)</b>	12 strips
<b>4.1.2 Anti-Human IgG Enzyme Conjugate</b> 1.0 ml each, concentrate.	2 vials
<b>4.1.3 Sample Diluent</b> 25 ml, concentrate.	1 bottle
<b>4.1.4 Conjugate Diluent</b> 10 ml, ready for use.	1 bottle
<b>4.1.5 Positive Control</b> 1.5 ml, ready for use.	1 vial
<b>4.1.6 Negative Control</b> 1.5 ml, ready for use.	1 vial
<b>4.1.7 Substrate Solution</b> 20 ml, ready for use.	1 bottle
<b>4.1.8 Washing Buffer</b> 20 ml, concentrate.	1 bottle
<b>4.1.9 Stopping Solution (1N NaOH)</b> 6.0 ml, ready for use.	1 vial

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## **Material required but not provided**

- Distilled or deionized water.
- Absorbent paper towels to blot dry the strips after washing steps and parafilm plastic wraps to cover strips during incubations.
- Suitable sized glass tubes for serum dilution.
- Micropipet with disposable tips to deliver 25  $\mu$ l, 50  $\mu$ l and 100  $\mu$ l.
- A microtiter plate washer or a squeeze bottle for washing.
- 10 ml pipettes for substrate buffer and conjugate diluent delivery.
- A 500 ml graduate cylinder.
- Microtiter plate reader with 405 nm absorbance capability.

### **4.2 Storage and Stability of the Kit**

Do not freeze test reagents, store all kit components at 4-8 °C at all times. Do not mix reagents from different kits. Do not allow reagents to stand at room temperature for extended periods of time. Store substrate tablets in the dark in an air tight container.

### **4.3 Preparations of Reagents**

Allow all reagents and required number of strips to reach room temperature prior to use.

#### **4.3.1 Anti-Human IgG Enzyme Conjugate**

Accurately transfer 5 ml of the Conjugate Diluent into one dropper bottle containing Anti-Human IgG enzyme conjugate (concentrate). Close the bottle and mix thoroughly by inversions. Each of the two conjugate (concentrate) bottles is sufficient for 6 strips. Reconstitute as needed.

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### **4.3.2 Sample Diluent**

The concentrated Sample Diluent, when stored at 4-8 °C, may have salt crystals at the bottom of the vial. Transfer the entire contents into 200 ml of distilled/deionized water in a 250 ml container (rinse out all crystals). Mix thoroughly, label the container as diluted Sample Diluent.

### **4.3.3 Washing Buffer**

Salt crystals may appear at the bottom of the Washing buffer concentrate vial during storage at 4-8 °C. Transfer the entire contents into 480 ml of distilled/deionized water in a 500 ml container (rinse out all crystals). Mix thoroughly and label the container as diluted Washing Buffer.

## **4.4 Storage and Stability of prepared Reagents**

### **4.4.1 Anti-Human IgG Enzyme Conjugate**

Store the diluted conjugate at 4-8 °C at all times. Record the date of reconstitution on the label. This diluted reagent expires 30 days after reconstitution.

### **4.4.2 Sample Diluent**

Store the diluted Sample Diluted at 4 - 8 °C. The diluted reagent is stable until the expiration shown on the vial.

### **4.4.3 Washing Buffer**

Store the diluted Washing Buffer at 4 - 8 °C. The diluted reagent is stable until the expiration shown on the vial.

## **4.5 Disposal of the Kit**

The disposal of the kit must be made according to the national official regulations. Special information for this product are given in the Material Safety Data Sheets (see chapter 3).

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## 4.6 Damaged Test Kits

In case of any severe damage of the test kit or components, IBL have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be use for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## 5. Specimen

### 5.1 Collection

#### Serum

Collect 5-10 ml of blood by venipuncture into a clot (red top) tube. Separate serum by centrifugation. Serum samples may be stored at 2-8 °C. Excessive hemolysis and presence of large clots or microbial growth in the test specimen may interfere with the performance of the test.

### 5.2 Storage

#### Serum

The serum samples obtained should be applied in the assay after storing for up to 24 hours at 2 - 8°C. If stored for longer periods the samples should be stored in aliquots at –20°C. Repeated freeze-thaw cycles have to be avoided.

### 5.3 Pretreatment

After removing assay reagents from the refrigerator, allow them to reach room temperature before pipetting. **Sample preparation** should be performed **in glass tubes**.

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## Dilution of samples

- Accurately pipet **25 µl of sample** into **2.5 ml** of the diluted **Sample Diluent** into an already labelled glass tube.
- Mix thoroughly.

## 6. Assay Procedure

### GENERAL REMARKS:

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.

Once the test has been started, all steps should be completed without interruption.

Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

**IMPORTANT NOTE:** Incubation temperatures varying by greater than  $\pm 1$  °C will definitely affect results.

### 6.1 Assay Procedure

**6.1.1** Assemble the number of **strips** needed for a test run in the **holder** provided. The microwell strip must be snapped in place firmly with its square side sitting on the numerical side of the holder or it will fall out and break.

**6.1.2** Familiarise yourself with the **indexing system** of wells, e.g. A1, B1, C1, D1, etc.

**6.1.3** Dispense **100 µl of Negative Control** into microwells C1 and D1.

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- 6.1.4** Dispense **100 µl** of **Positive Control** into microwells E1 and F1.
- 6.1.5** Add **100 µl** of diluted **patient serum** (see 5.3) to microwells G1 and H1. For more patient samples, use additional strips and add diluted samples to microwells in **duplicate**. There should be 100 µl of solution in each microwell to be assayed except A1 and B1 which are empty at this point and will be used later.
- 6.1.6** Any well **strips not used** should be stored in the ziplock bag provided for the next run.
- 6.1.7** **Cover** the plate with a parafilm/plastic wrap (to prevent contamination) and leave for **1 h at room temperature (25 ± 1 °C)**.
- 6.1.8** After incubation, **decant** the solution from all wells. Blot the plate dry by tapping gently on a paper towel. If an automatic plate washer is being used, wash each well with **300 µl** (0.3 ml) of the diluted **Washing Buffer**. When a squeeze bottle is used, fill the wells with diluted Washing Buffer carefully and then drain the buffer from the microwells. **Repeat** the procedure **two more times** and blot dry the plate with a paper towel. Avoid air bubbles in the well during washing.
- 6.1.9** Add **100 µl** of **Anti-Human IgG Enzyme Conjugate** (see 4.3.1) to all microwells except wells A1 and B1.
- 6.1.10** **Cover** the plate with a parafilm/plastic wrap and let it stand at **room temperature (25 ± 1 °C) for one hour**.
- 6.1.11** After incubation, **repeat** the **washing** step (see 6.8) and blot dry the microwells.
- 6.1.12** Add **100 µl** of **Substrate Solution** to all microwells including wells A1 and B1. Be sure to dispense the substrate solution at a **rapid steady pace** without any interruption.
- 6.1.13** Cover the plate and leave the plate in the **dark** for **30 minutes** at **25 °C ± 1 °C**.

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**6.1.14** After 30 minutes promptly add **50 µl** of the **Stopping Solution** into each well.

**6.1.15** Set up the **microtiter plate reader** to read at **405 nm** absorbance according to manufacturer's instructions. Blank the plate reader with well A1 or B1 and read the absorbance at 405 nm.

**6.1.16** Calculate the data according to the following section.

## **6.2 Calculation of Results**

Record the spectrophotometric readings [optical density (O.D.) in absorbance units] as shown in the example report form (see page 13). The actual O.D. reading from your assay may be different.

- Calculate the average reading of a sample or control done in duplicate.
- The average reading of the negative control data is N, positive control data is P, and sample data is S.
- Calculate the cut-off point of each run:

$$\text{Cut-off Point X} = N \times 2,5$$

Enter either (+) or (-) by comparing the average sample O.D. value (S) with the calculated cut-off point value as shown in the example below. Note that the “cut-off point” is only to be used as a reference point and is an arbitrary value.

## Report Form (Sample)

Kit-/Lot-No. \_\_\_\_\_ Expiration Date: \_\_\_\_\_

Assay Date: \_\_\_\_\_ Operator: \_\_\_\_\_

### Section A: Control Results

	Data		Cut-off Value X	Result
Controls	O.D.	Average O.D.	$X = (2.5 \times N)$	
Negative	0.187			
Control	0.193	N = 0.190	X = 0.475	
Positive	1.190			
Control	1.270	P = 1.230		Positive

### Interpretation:

1. For a valid test,  $N \leq 0.60$  and  $P \geq X$ . Repeat the test if results are not valid.
2. Positive Result: Average Sample O.D. (S) > X.
3. Negative Result: Average Sample O.D. (S)  $\leq$  X.

### Section B: Patient Sample Results

Patient	Data		Results (Cut-off Point: X = 0.475)	
	O.D.	Average O.D.	Positive (+) (Average O.D. > X)	Negative (-) (Average O.D. < X)
1	0.71			
	0.75	$S_1 = 0.73$	+	
2	0.31			
	0.33	$S_2 = 0.32$		-

Date: \_\_\_\_\_

Reported by: \_\_\_\_\_

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## **7. Assay characteristics**

### **7.1 Specificity**

The specificity of antigen coated microwell strips was established by Western blot analysis using confirmed positive samples for IgG to Islet Cell antigen. Patients with thyroid autoantibodies and rheumatoid factors read negative on ICA ELISA.

### **7.2 Accuracy**

#### **7.2.1 Quality Control**

Positive and negative kit controls must be run along with unknown samples each time for results to be valid. The negative control should show an optical density (O.D.) value not more than 0.600 and the positive control should show an O.D. equal to or greater than the cut-off point (X). If the values are beyond this range, the test should be repeated.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or IBL-International directly.

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## **8. Limitations of Use**

### **8.1 Interfering Substances**

Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen - Collection'.

Azide and thimerosal at concentrations higher than 0.1 % interfere in this assay. Therefore control sera or samples containing higher concentrations of the above mentioned components may give false results.

### **8.2 High-Dose-Hook Effect**

There exists no High-Dose-Hook effect in a competitive assay. In case of sandwich assays the probability of a High-Dose-Hook effect is reduced if the antibody and antigen containing solutions are added in a sequential order.

### **8.3 Limitations and sources of error**

Although a higher ICA titer will produce a higher O.D. reading, the test is designed for qualitative determination of ICA only.

Poor test reproducibility may result from:

- Inconsistent delivery of drops;
- Improper storage of reagents;
- Improper reconstitution of reagents;
- Incomplete washing of microwells;
- Substrate reagent old or exposed to light;
- Unstable/defective spectrophotometer; and/or
- Error in following the assay procedure.

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## 9. Legal Aspects

### 9.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

### 9.2 Complaints

Complaints will only be accepted in written format (preferably on the manufacturer's complaint form) and only if all details of the test kit, as well as the test results, are included. A copy of the complaint form is available from IBL-International upon request.

### 9.3 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

Save the weak positive and borderline samples (within 5 % above the cut-off point) and store at -20 °C. Fresh samples from these patients should be tested again every six months together with the previous serum samples.

**This is a screening test only. The diagnosis of IDDM should be made with data from the patient's medical history, clinical symptoms, and results of other tests.**

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## **9.4 Liability**

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject to point 9.3. are also invalid. 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 test kit during transportation is not subject to the liability of the manufacturer.

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- duration and type of diabetes, coexistent autoimmune disease and HLA type. *Diabetes*, 26:138-147.
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	<b>Islet Cell Autoantibody (ICA) ELISA</b>	<b>Cat. No.</b>	<b>NM 590 81</b>
	Short Instructions for Use	Size:	12 x 8
		Storage:	2 - 8 °C

**1. Preparation of Reagents** (Volumens stated for 48 determinations)

- **Anti-Human IgG Enzyme Conjugate:** Accurately transfer 5 ml of the Conjugate Diluent into one dropper bottle containing Anti-Human IgG enzyme conjugate (concentrate). Close the bottle and mix thoroughly by inversions. Store the diluted conjugate at 4-8 °C at all times. Record the date of reconstitution on the label. This diluted reagent expires 30 days after reconstitution.
- **Sample Diluent:** The concentrated Sample Diluent, when stored at 4-8 °C, may have salt crystals at the bottom of the vial. Transfer the entire contents into 200 ml of distilled/deionized water in a 250 ml container (rinse out all crystals). Mix thoroughly, label the container as diluted Sample Diluent. Store the diluted Sample Diluted at 4 - 8 °C.
- **Washing Buffer:** Salt crystals may appear at the bottom of the Washing buffer concentrate vial during storage at 4-8 °C. Transfer the entire contents into 480 ml of distilled/deionized water in a 500 ml container (rinse out all crystals). Mix thoroughly and label the container as diluted Washing Buffer. Store the diluted Washing Buffer at 4 - 8 °C.

**2. Specimen Collection and Storage**

Samples: Serum

Storage: for up to 24 h at 2 - 8 °C, longer storage at -20°C. Avoid repeated freezing and thawing.

**3. Assay Procedure**

Allow reagents to reach room temperature. Unused reagents should be stored at 2-8°C.

**NOTE:** Incubation temperatures varying by greater than  $\pm 1$  °C will definitely affect results.

- **A: Dilution of Samples** (in Glass Tubes)

✦	Pipet <b>2.5 ml of Sample Diluent</b> into each labelled tube.
✦	Add <b>25 µl of Serum Sample</b> into the respective tubes. Mix thoroughly.

• **B: ELISA**

✦	Pipet <b>100 µl of Negative Control, Positive Control and diluted Samples</b> . Let two wells empty for substrate blank.
📄	Seal plate and incubate for <b>1 h at room temperature (25 ± 1 °C)</b> .
🌀	Empty plate, <b>wash 3 x with 300 µl of diluted Washing Buffer</b> , eliminate residual fluids. *
✦	Pipet <b>100 µl of Anti-Human IgG Enzyme Conjugate</b> (except wells for blank).
📄	Seal plate and incubate for <b>1 h at room temperature (25 ± 1 °C)</b> .
🌀	Empty plate, <b>wash 3 x with diluted Washing Buffer</b> , eliminate residual fluids.*
✦	Pipet <b>100 µl of Substrate Solution</b> into all wells (including wells for blank).
📄	Seal plate and incubate for <b>30 min. at room temperature (25 ± 1 °C) in the dark</b> .
📄	Pipet <b>50 µl of Stopping Solution.**</b>
📄	Briefly mix contents, read the optical density at <b>405 nm</b> (reference wave length 600-650 nm) <b>within 60 min.</b> after stopping.

\* Wash procedure is essential for the assay results.

\*\* Stopping solution should be pipetted after incubation in the same time intervals as the substrate reagent.

Calculate the average reading of a sample of control done in duplicate.

The average reading of the negative control data is N, positive control data is P, and sample data is S.

Calculate the cut-off point of each run:














$$\text{Cut-off Point } X = N \times 2,5$$

Enter either (+) or (-) by comparing the average sample O.D. value (S) with the calculated cut-off point. Note that the "cut-off point" is only to be used as a reference point and is an arbitrary value. The borderline cases (within 5 % above the cut-off point) should be tested again every 6 months with the previous serum sample as reference. These samples should be stored at -20 °C, or below, until the next test run.

**Attention:** For comprehensive information about the test procedure please refer to the detailed instructions for use available on the Internet or upon request from IBL in Hamburg.

June 11<sup>th</sup> 2003/K1/L1

# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	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:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	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: / Fabbicante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>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.</p>	

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**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). 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.