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Instructions for Use

IRT Neonatal
Luminescence Immunoassay

Luminescence Immunoassay for the quantitative determination of immunoreactive trypsin (IRT) in human newborn blood spot samples.

REF **RE68015 / RE68019**

Σ **480 / 2400**

2°C 8°C

EU: IVD CE

IBL International GmbH
Flughafenstrasse 52a
22335 Hamburg, Germany

Always there for you

1. INTENDED USE

Luminescence Immunoassay for the quantitative determination of immunoreactive trypsin (IRT) in human newborn blood spot samples.

For neonatal screening of cystic fibrosis (CF).

2. SUMMARY AND EXPLANATION

Cystic fibrosis (CF) is one of the most common autosomal recessive diseases caused by mutations in the CF transmembrane conductance regulator gene (CFTR). CF can result in death at an early age, primarily from progressive lung disease, although a number of organs are often involved. CF occurs at an incidence of approximately 1:2000 - 1:5000 live births in Europe and North America. The first European experiences in cystic fibrosis (CF) newborn screening (NBS) date back to the early nineteen seventies, with pioneering programmes examining the albumin content of meconium. The elevation of Immunoreactive Trypsin (IRT) in the blood of neonates with CF and its measurement in dried blood spots was first described in 1979. During the following decade, the determination of IRT levels in heel blood was introduced in several countries. Further improvement was possible after cloning of the CFTR gene in 1989 and subsequent identification of common population specific CFTR gene mutations allowed inclusion of DNA testing into screening protocols. Studies have shown that early diagnosis of CF through neonatal screening, combined with aggressive nutritional therapy, can significantly enhance long-term nutritional status. The two most common protocols for CF screening are (a) measuring IRT on one sample and then on a second sample, and (b) IRT testing followed by DNA mutation analysis on the same sample [1, 2, 3].

3. TEST PRINCIPLE

In the procedure, a disk is punched from a blood spot collected on filter paper. This disk is placed into the antibody well along with an Assay Buffer containing the enzyme conjugated specific anti-trypsin 1 and antitrypsin 2 antibodies. After incubation, the Assay Buffer and blood spot are aspirated out, the well is washed and chemiluminescence substrate is added to the well. The RLU is measured after 45 minutes. A standard curve is then constructed from which unknown concentrations of trypsin can be calculated.

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. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
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. For this reason reagents should be treated as potential biohazards in use and for disposal.
10. 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.

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 sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is opened. Make sure that the opened bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Blood Spots

Blood from the newborn’s heel should be collected only from the medial or lateral section of the plantar surface. The usual precautions for blood collection should be observed. After puncture of the heel the first drop of blood should be wiped away with a sterile gauze.

Touch the collection card against a large hanging drop of blood and allow a sufficient quantity of blood to soak into the filter paper in one step and so to fill the pre-printed circle completely. Repeat the procedure to fill the required number of pre-printed circles on the collection card.

Allow the blood spots to air-dry for 3 hours at room temperature away from direct sunlight.

⚠	Because the standards are established with filter cards from Schleicher & Schuell No. 903 and there is a significant influence of the results by the filter card material (see LIMITATIONS OF THE PROCEDURE), it is recommended to use these cards also for the patient samples. Don’t squeeze the puncture site during the collection because this will cause hemolysis or dilution of the blood with tissue fluid. Don’t apply successive drops of blood to the same pre-printed circles. Don’t touch or smear the blood spots. Take care that the blood spot samples are visually okay (e.g. no blood smears, no coagulates, no finger-prints on the spots).
⚠	Cystic fibrosis screening does not require a change in current blood sampling practice. Most reported collection point of time is 4th or 5th day after birth [2]. National and country specific guidelines to sample collection point of time must be considered.

Storage:	2-8 °C	Keep away from heat or direct sunlight.
Stability:	up to 4 months [1]	

7. MATERIALS SUPPLIED

RE68015	RE68019	Symbol	Component
5 x 12 x 8	25 x 12 x 8	MTP	**Microtiter Plate** White colored. Coated with anti-human Trypsin antibodies (rabbit).
1 x 350 µL	5 x 350 µL	ENZCONJ CONC	**Enzyme Conjugate Concentrate** (321x) Contains: antibody alkaline phosphatase conjugate.
1 x 6 x 15 DBS	2 x 6 x 15 DBS	CAL A-F	**Standard A-F** Ready to use. Contains: human blood. Schleicher & Schuell paper No. 903. 15 Blood Spots / card. Concentrations see labels or QC certificate.
1 x 2 x 15 DBS	4 x 2 x 15 DBS	CONTROL 1+2	**Control 1+2** Control 1: middle Control 2: high Ready to use. Contains: human blood. Schleicher & Schuell paper No. 903. 15 Blood Spots / card. Concentrations / acceptable ranges see labels or QC certificate.
1 x 6 mL	4 x 6 mL	ASSAYBUF CONC	**Assay Buffer Concentrate** (20x) Contains: PBS Buffer, Tween, Protease Inhibitor, casein, preservatives.
5 x 5.5 mL	6 x 21.5 mL	LUMINREAG	**Chemiluminescence Reagent AP** Ready to use.
2 x 100 mL	4 x 100 mL	WASHBUF CONC	**Wash Buffer Concentrate** (10x) Contains: Tris Buffer, Tween, < 0.1 % NaN_3.
10 x	30 x	FOIL	**Adhesive Foil** black

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50; 100-500; 1000 µL
2. Orbital shaker (400-600 rpm)
3. Vortex mixer
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Disc remover (optional) – device for removal of eluted filter paper discs
7. Luminescence Immunoassay-Reader (e.g. Berthold microtiter plate luminometer)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer
10. Blood collection cards (Schleicher & Schuell 903 recommended)
11. Blood spot puncher, 3 mm (e.g. Sauer, Hannover, Germany)
12. Microtiter Plate (round bottom), only needed for the alternative 2-step procedure with external dilution. This material can be ordered separately from IBL under REF ZL650101.

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. The following precautions should be taken when dried blood spots for the determination are punched:
    1. Punch the discs only from uniformly-covered dried blood spots.
    2. For double determinations, use only two nearby discs from one blood spot only, in order to avoid a chromatographic effect as much as possible.
    3. Note: Do not punch too close to the edge of the blood spot (1 mm away from the edge)!
8. Check the blood spot samples are visually okay (e.g. no blood smears, no coagulates, no finger-prints on the spots).
9. Unused wells/tubes and dried blood spots should be returned immediately to the resealed pouch, including the desiccant.
10. Ensure all dried blood spots, without exception, are removed from the microtiter plate before starting washing.

10. PRE-TEST SETUP INSTRUCTIONS

Preparation of concentrated components (volumes below are stated for 1 Plate)

Allow the reagents to reach room temperature and mix them thoroughly before preparing the solutions. Possibly existing precipitates in assay buffer will dissolve when liquid equals room temperature.

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	WASHBUF CONC	Up to 150 mL	bidist. water	1:10	Mix vigorously.	2-8 °C	4 weeks
800 µL	ASSAYBUF CONC	Up to 16 mL	bidist. water	1:20	Mix without foaming.	2-8 °C	24 hours
50 µL	ENZCONJ CONC	with 16 mL	Assay Buffer diluted	1:321	Mix without foaming.	Prepare freshly and use only once.	

11. TEST PROCEDURE

11.1. MAIN TEST PROCEDURE (1-STEP-PROCEDURE)

1.	Punch **one 3 mm** blood soaked filter paper spot of each **Standard, Control and sample** into the respective wells of the **coated** microtiter plate. Standards and Controls should be set up in duplicates. **Note: Do not punch too close to the edge of the blood spot!**
2.	Pipette **150 µL** of **freshly prepared Enzyme Conjugate** into each well. Be certain all blood spots are submerged in Enzyme Conjugate.
3.	Cover plate with black adhesive foil. Incubate the blood spots **2 hours** at **RT (18-25 °C)** on an orbital shaker (400-600 rpm).
4.	Remove adhesive foil carefully. Flick of contents of all wells. Tap plate dry to remove remaining liquids. Alternative remove the discs with a disc remover. Wash **3-5 times** with **250-300 µL** diluted **Wash Buffer.**
5.	Pipette **50 µL** of **Chemiluminescence Substrate** into each well. Cover plate with black adhesive foil. Incubate at **RT (18-25 °C)** on an orbital shaker (400-600 rpm) for **45 min.**
6.	Remove adhesive foil carefully. **Measure** relative luminescence units (RLU) with a luminometer.

11.2. ALTERNATIVE TEST PROCEDURE (2-STEP-PROCEDURE; EXTERNAL ELUTION)

1.	Punch **one 3 mm blood** soaked filter paper spot of each Standard, Control and sample into the respective wells of the **uncoated** round-bottom microtiter plate. Standards and Controls should be set up in duplicates. **Note: Do not punch too close to the edge of the blood spot!**
2.	Pipette **150 µL** of **freshly prepared Enzyme Conjugate** into each well. Be certain all blood spots are submerged in the Enzyme Conjugate.
3.	Elute the blood spots **30 min** at **RT (18-25° C)** on an orbital shaker (400-600 rpm).
4.	Transfer **100 µL** of the liquid to the **coated** microtiter plate (8-Channel transfer pipette). Avoid touching the spots with the tips of the pipette!
5.	Cover plate with black adhesive foil. Incubate **2 hours** at **RT (18-25 °C)** on an orbital shaker (400-600 rpm).
6.	Remove adhesive foil carefully. Flick of contents of all wells. Tap plate dry to remove remaining liquids. Wash **3-5 times** with **250-300 µL** diluted **Wash Buffer.**
7.	Pipette **50 µL** of **Chemiluminescence Substrate** into each well. Cover plate with black adhesive foil. Incubate at **RT (18-25 °C)** on an orbital shaker (400-600 rpm) for **45 min.**
8.	Remove adhesive foil carefully. Measure relative luminescence units (RLU) with a luminometer.

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 comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. 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.

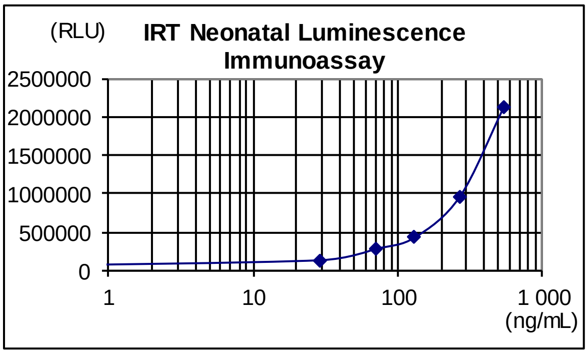
13. CALCULATION OF RESULTS

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

Typical Calibration Curve

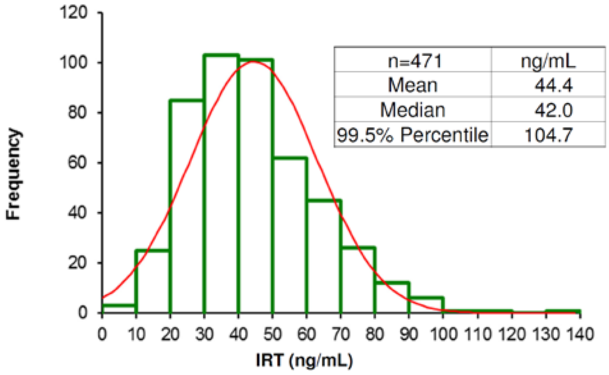
(Example. Do not use for calculation!)

Standard	Trypsin (ng/mL)	RLU	RLU/RLUmax (%)
A	0	46114	2%
B	29	131159	6%
C	71	280227	13%
D	131	429931	20%
E	268	963509	45%
F	539	2131366	100%



14. EXPECTED VALUES

Based on the assumption that the expected values of immunoreactive trypsin (IRT) follow a normal distribution, it is common to use floating cut-off because of the variations in IRT levels (e.g. ethnic or seasonal variations). There are several options for an initial threshold based on percentile values of the normal distribution measured in single determinations (e.g.97th, 99th or 99.4th percentile values). Sample values above the determined cut off (positive) have to be repeated in duplicates. As an example for normal distribution the results of 471 randomly selected newborns measured with the IBL IRT neonatal screening luminescence immunoassay (1-step-procedure) are shown:



Depending on the application of samples of different populations of newborns it is highly recommended that each laboratory establishes its own range of normal values and that this distribution of values is coordinated with the recommendations of the responsible society of this geographic region.

Both test procedures indicated (1-step and 2-step with external elution) are not exchangeable. Therefore, the expected values should be established with the procedure chosen for further screening. It is recommended that each laboratory establishes its own range of normal 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.

15. LIMITATIONS OF THE PROCEDURE

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

Any result with an elevated concentration has to be indicated as ‘presumptive positive’ and has to be confirmed with further sampling and testing.

A false negative result of this assay cannot be excluded with absolute certainty.

It is recommended to use S&S 903 filter cards for the blood spot samples.

Conditions which are known to cause anomalous analytical assay results are:

- sample spot not uniformly saturated with blood
- sample spots punched too close to the edge of the blood spot
- poorly collected and improperly dried specimens
- non-eluting blood spot due to deterioration of sample caused by exposure to heat and humidity
- contamination of blood spot filter paper with fecal material

For cross-reactivities, see PERFORMANCE.

The following blood components do not have a significant effect on the test results up to the below stated concentrations:

Bilirubin	5 mg/mL
Triglyceride	91 mg/mL

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	The following substances showed a cross reactivity lower than <0.01 %: Pepsinogen II PGC; alpha2-Macroglobulin; alpha-1-Antitrypsin; alpha-Chrymotrypsin; gamma-Globulin			
Analytical Sensitivity (Limit of Blank)	0.3 ng/mL	Mean signal (Zero-Standard) + 2SD		
Precision	Range (ng/mL)	CV (%) (1-Step Proc.)	Range (ng/mL)	CV (%) (2-Step Proc.)
Intra-Assay	57-205	6.7-9.2	62-223	5.8-12.6
Inter-Assay	56-193	8.3-12.0	58-184	9.4-14.1
Inter-Lot	58-194	9.6-14.3	-	-
Method Comparison versus Methods / Assays	IBL-Assay LUM = 0.97 (IBL IRT neonatal screening ELISA)+13.3			r = 0.89; n = 376
	IBL-Assay LUM = 1.42 (IRT Fluorometric Assay) + 17.1			r = 0.79; n = 471
	IBL-Assay LUM = 0.86 (IRT Colorimetric ELISA) + 8.86			r = 0.89; n = 169

17. PRODUCT LITERATURE REFERENCES

1. Li L et al. Development and characterization of dried blood spot materials for the measurement of immunoreactive trypsinogen. J Med Screen 13:79 - 84 (2006)
2. Castellani C et al. European best practice guidelines for cystic fibrosis neonatal screening. Journal of Cystic Fibrosis 8 153 - 173 (2009)
3. Crossley JR et al. Dried-blood spot screening for cystic fibrosis in the newborn. Lancet 1: 472 - 4 (1979)
4. Stopsack M et al. Neonatal screening for cystic fibrosis. Pros and cons. Monatsschr Kinderheilkd (2009)
5. Salvatore D et al. An overview of international literature from cystic fibrosis registries 2. Neonatal screening and nutrition/growth. Review. Journal of Cystic Fibrosis (2009)
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7. Heeley A F et al. Screening for cystic fibrosis by dried blood spot trypsin assay. Archives of Disease in Childhood, 57, 18 - 21 (1982)
8. Rodrigues R et al. Cystic fibrosis and neonatal screening. Review. Cad. Saúde Pública, Rio de Janeiro, 24 Sup 4, 475 - 484 (2008)

Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
BIO	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
BIO	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
UDI	Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
i	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / 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 / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / 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: / Armazenar em: / Αποθήκευση στους:
2-8°C	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / 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. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. 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.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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