Instructions for Use

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# BetaPrion® HUMAN ELISA

**Enzyme immunoassay for quantitative determination of human prion protein in CSF other cell free biological samples.**

REF **30227871**

Σ **96**

**For illustrative purposes only.**
**To perform the assay the instructions for use provided with the kit have to be used**

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Always there for you

# Contents



# Introduction

## Intended use

The BetaPrion HI ELISA is designed to detect and quantify the level of total prion protein in human cerebrospinal fluid (CSF) and other cell free biological samples (e.g. plasma) All contents of the BetaPrion are produced under the guidelines of quality control accordingly to the DIN EN ISO 13485 requirements.

The BetaPrion HI ELISA is for research use only and not intended as a diagnostic test.

## Warranty and technical support

The manufacturer guarantees the correct functioning of the kit for the applications described in the instructions for use (IFU). During the warranty pe- BetaPrion HUMAN ELISA allows for precise and reproducible data collection in connection with superior sensitivity. Any warranty claims shall only be valid if the general principles of Good Laboratory Practice (GLP) and the manufacturer's recommendations are observed.

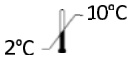
To improve the application and design, Roboscreen GmbH reserves the right of product replacement or modification. The manufacturer may be contacted at any time for questions and problems or technical support concerning the quantification of human prion protein.

CONSULT INSTRUCTIONS FOR USE

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

**Notes on the use of this instructions for use**

easy reference and orientation, the IFU uses the following warning and information symbols as well as the shown methodology:

| | |
|---|---|
|  | Catalogue number |
|  | Contains sufficient reagents for <N> tests |
|  | Storage conditions |
|  | instructions for use |
|  | |
|  | Manufactured by |
|  | For single use only |

The following abbreviations are used in the IFU:

| | |
|---|---|
| | Alzheimer's disease |
| | Cerebrospinal fluid |
| | ·linked immunosorbent assay |
| | Laboratory Practice |
| | Horseradish peroxidase |
| | Instruction for use |
| | Monoclonal antibody |
| | Optical density |
| | Room temperature (18 |
| | Tetramethylbenzidine |

# 2 Safety precautions

We recommend reading this chapter thoroughly before using this kit, to ensure the safety of the user and error-free utilization.
Any safety instructions and additional information of this IFU must be observed at all times.

Read and make sure you understand the operating instructions completely and thoroughly before carrying out the test. Use the currently valid version from the kit.

Notify the respective supplier in writing within one week from receiving the merchandise, should the test pack be substantially damaged. Damaged components must not be used to carry out the assay, however, they should be kept until the transport damages are finally settled.

Comply with Good Laboratory Practice and safety regulations. Wear laboratory coats, disposable gloves and safety goggles whenever the need arises.

Reagents of this kit which contain hazardous substances may cause irritations to eyes and skin. See indications under *(5) Kit components, storage and expiry date* and on the labels. Safety data sheets of this product are available upon request.

Chemicals and prepared or used reagents shall be disposed of as hazardous waste in compliance with the respective national regulations.

The cleaning staff has to be instructed by experts with regard to any potential risks and the appropriate handling of such substances.

Avoid any contact with stop solution. This may cause irritations to the skin and chemical burns.

**FOR SINGLE USE ONLY!**

This kit is made for single use only!

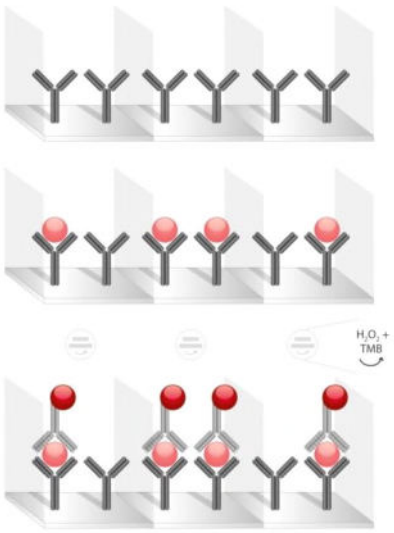
**ATTENTION!**

Do not eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

# 3 Test principle

The BetaPrion HUMAN ELISA is based on a sensitive sandwich ELISA using two specific monoclonal anti-human prion protein antibodies. The capture antibody, which is immobilized on the surface area of the microtiter plate, specifically recognizes the conformational epitope of human prion protein. Prion protein from human CSF, serum or plasma samples, standards and controls bound by the capture antibody is detected by a HRP-conjugated mAb that specifically binds to amino acids 151-180 (RYYRENMHRY) of human prion protein (Dorey, et al., 2015). Amount of bound conjugated antibody is estimated using chromogenic substrate tetramethylbenzidine (TMB). The concentration of prion protein is proportional to the obtained optical density. Controls are included for the proof of reproducibility and evaluation of the assay within labs.




1. Ready to use: Capture antibody coated on well plate

2. Binding of human prion protein by capture antibody.

3. Detection of bound prion protein by HRP-conjugated antibody specific for human prion protein.

# 4 Performance assessment

Assay precision, sensitivity, selectivity and reproducibility were assessed by analyzing 225 human CSF samples from patients with symptomatic prion disease, presymptomatic prion disease mutation carriers, patients with non-prion dementia, and patients with normal pressure hydrocephalus, as well as other non-prion controls across 41 plates (Table 1).

**Table 1: The technical performance of the BetaPrion HUMAN ELISA supports reliable quantification of PrP in human CSF** (Vallabh, et al., 2019)

| Experiment | Results |
|---|---|
| Within-plate technical replicate reproducibility (same dilution) | CV = 8% |
| Within-plate technical replicate reproducibility (all dilutions) | CV = 11% |
| Between-plate technical replicate reproducibility | CV was 22% in an interplate control sample run on 17 plates on different days |
| Sensitivity | LLOQ is 3–5× the blank signal |
| Selectivity | Nonereactive for recombinant mouse PrP, rat CSF, and cynomolgus monkey CSF, artificial CSF, and protease digested CSF. |
| Dilution linearity | Linear across two samples and five dilutions. |
| Standard curve reproducibility | CV was <10% at all six nonzero standard curve points across five replicates |

CV= coefficient of variation; LLOQ= lower limit of quantification

## 4.1 Linearity of dilution

Consistent dilution linearity was observed within the assay's stated dynamic range of 1 – 20 ng/mL PrP, providing reassurance that this technique can be used to compare PrP levels across samples even when these levels differ by one log (Figure 1A). Five replicates of the kit's internal six point standard curve, reconstituted from lyophilized standards, were run in parallel on one plate. Across the dynamic range of the assay, the coefficient of variation falls below

10% for all points and well below the 20% FDA recommended limit in standard variability for ligand-binding assays (Vallabh, et al., 2019).

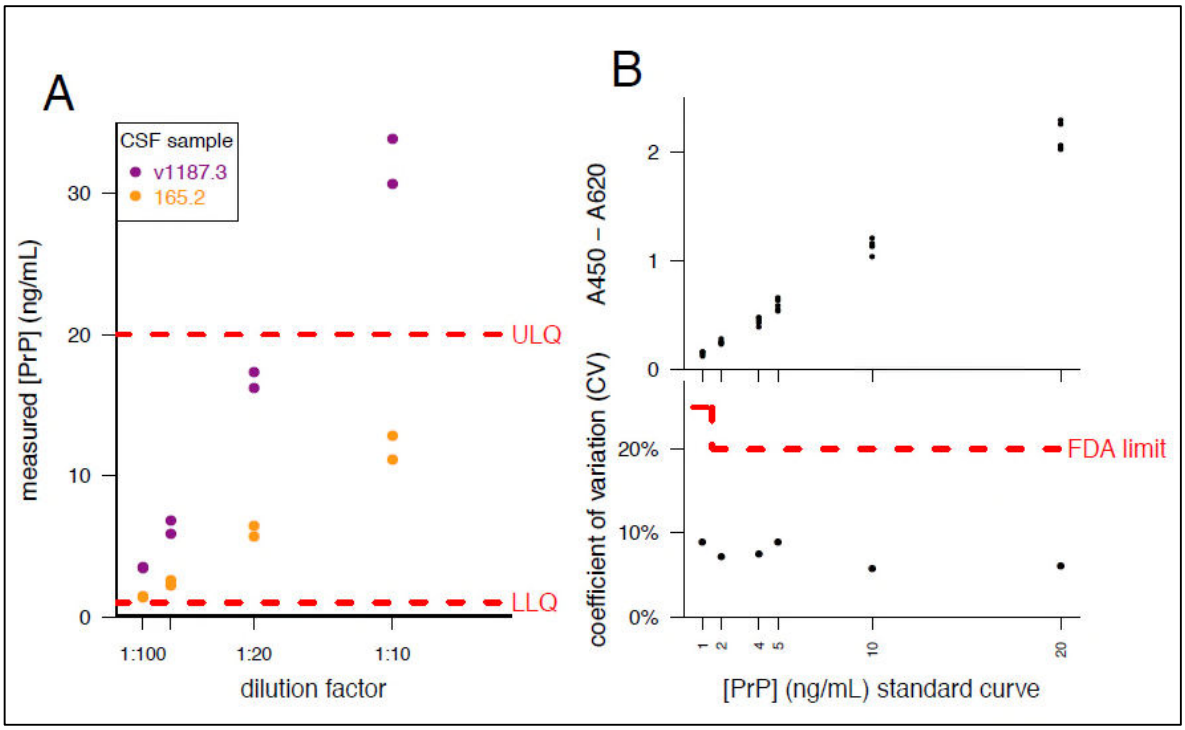



**Figure 1: The BetaPrion HUMAN ELISA quantifies PrP in a technically reproducible and sensitive manner** (Vallabh, et al., 2019)**. A)** Consistent dilution linearity was observed within the assay's stated dynamic range of 1 – 20 ng/mL PrP. Purple and yellow dots represent two different samples measured in duplicate at each of four dilutions. **B)** Standard curve reproducibility.

## 4.2 Performance data for blood serum samples

The assay performance for measurement of human prion protein in peripheral blood serum samples was assessed during pre-tests for a large study investigating the association of prion protein levels and cognitive functioning in human (Breitling, et al., 2012). Detection limit of the assay was 0.132 ng/ml and blinded double measurements of 20 samples supported a high precision with a Spearman correlation of 0.97 between the measurements.

# it components, storage and expiry date

## Kit components

| Component | Σ | Description |
|---|---|---|
| | | Coated immu strips containing anti-man prion protein antibody, blocked and stabilized. Ready to use. |
| X Wash buffer | | X Wash buffer containing . detergent . |
| | | Lyophilized prion protein standards for preparing a standard curve for quantification of human prion protein biological Containing PBS, protein and proclin 300. |
| | 3 | prion protein |
| | 3 | prion protein |
| | 3 | prion protein |
| | 3 | prion protein |
| | 3 | prion protein |
| | 3 | prion protein |
| Negative Control | 1 m | Negative control (blank), containing - Ready to use. |
| conjugate | 1 m | conjugated mAb anti-human prion protein, 20X concentrate, containing PBS, protein, detergent and proclin 300. |
| Assay buffer | | buffer containing PBS, protein, detergent and proclin 300. Ready to use. |

## Kit components, storage and expiry date

| Component | Σ | Description |
|---|---|---|
| Control high |  | Lyophilized prion protein high positive control (CTRL), containing PBS, protein and proclin 300. |
| Control low |  | Lyophilized prion protein low positive control (CTRL), containing PBS, protein and proclin 300. |
| Staining solution | | TMB/peroxide solution. Ready to use. |
| Stop solution | | 1 M sulphuric acid. Ready to use. |
| Blocking solution | | locking solution for reduction of unspecific reactions to of the assay. |
| Sealing tape | | |
| Instruction for use | | |

### 5.2 Storage and expiry date

The kit is delivered at ambient temperature and should be stored at 6 ± 4 °C. Protect from heat and direct sunlight. Under these conditions, the kit has a shelf life as indicated on the kit box while retaining its endurance and stability.

Prepared kit components have the following expiry dates:

| Component | Preparation step | Expiry date |
|---|---|---|
| D1 | Coated immune strips after opening of bag, taking out strips and closing of bag. | At 6 ± 4 °C up to 4 weeks. |
| D2 | Ready to use 1X wash solution. | At 6 ± 4 °C up to 1 week. |
| D3.1-D3.6 | Standards D3.1-D3.6 dissolved in D6. | At 6 ± 4°C up to 4 h. |
| D7, D8 | Controls D7 and D8 dissolved in D6. | At 6 ± 4°C up to 4 h. |
| D5 | Ready-to-use HRP-conjugate 1:20 diluted. | At 6 ± 4°C up to 4 h. |

# 6 Components not included in the kit:

- Calibrated micropipettes with CV < 3 %
  Volume: 0.5-10 µl; 10-100 µL; 100-1000 µL
- Calibrated 8-channel micro-pipette with reagent reservoirs
- Vortex mixer
- Automated or semi-automated ELISA plate washing system
- Bidistilled or deionized water
- Paper towels, pipette tips and timer
- ELISA plate reader for reading absorbance at 450 and 620 nm
- Polypropylene tubes for sample dilution
- Tubes (10-50 ml) for preparation of components

# 7 Preparation of components

Immune strips **D1**, negative control **D4**, assay buffer **D6**, staining solution **D9** and stop solution **D10** are **ready to use** components.

## 7.1 1X Wash solution

Mix 10X wash buffer **D2** by 2-3 x inversing and dilute **D2** with bidistilled or deionized water 1:10 as described below before the first wash step of the immunoassay.

| Number of Immune strips | Volume of 1X Wash solution | Volume of 10X Wash buffer D2 | Volume of bidistilled or de-ionized water |
|---|---|---|---|
| 1-4 | 300 ml | 30 ml | 270 ml |
| 5-8 | 600 ml | 60 ml | 540 ml |
| 9-12 | 900 ml | 90 ml | 810 ml |

## 7.2 Standards D3.1 – D3.6

Add **1.0 ml** of assay buffer **D6** to each standard vial **D3.1 – D3.6** and mix quickly, e.g. within 2 s by vortex.

## 7.3 Controls D7 and D8

Add **1.0 ml** of assay buffer **D6** to each control vial **D7** and **D8** and mix quickly, e.g. within 2 s by vortex.

## 7.4 1X Blocking solution

Mix 10X Blocking solution by 2-3 x inversing and dilute with assay buffer **D6** in ratio 1:10 before using it for sample dilution.

## 7.5 1X HRP conjugate

Dilute 20X HRP conjugate D5 at 1:20 ratio with assay buffer D6. Mix by shaking the tubes.

| Number of Immune strips | Volume of 20X HRP D5 | Volume of assay buffer D6 |
|---|---|---|
| 1 – 4 | 0.3 ml | 5.7 ml |
| 5 – 8 | 0.4 ml | 7.6 ml |
| 9 - 12 | 0.6 ml | 11.4 ml |

# 8 Procedure notes

Any improper handling of samples or modification of the test procedure may influence the results. The indicated volumes, incubation times, temperatures and pretreatment steps must be followed strictly regarding this instruction.

Be sure that required reagents, materials and devices are prepared ready at the appropriate time.

1X HRP conjugate D5, staining solution D9 and stop solution D10 should be transferred by 8-channel micropipette or a pipette with reservoir (multistep pipette) to all wells of the immune strips.

Washing should be done by 8-channel micropipette or ELISA plate washer. Avoid drying and over stressing of wells and control exact washing of all wells.

All measurements can be done in **single determinations**; however a dual determination increases the safety of the results and allows additional evaluations for the precision of the measurements. For duplicate determinations, $R^2$ of the standard curve should be ≥ 0.99.

It is recommended to use a pipetting scheme to apply all STD, CTRL and samples.

## 8.1 CSF specimen collection and storage

The Alzheimer's Biomarker Standardization Initiative provides the following recommendations for the pre-analytical and analytical aspects for AD biomarker testing in CSF (Vanderstichele, et al., 2012).

**Specimen collection**

Lumbar puncture may be performed at the vertebral body L3-L5 with the patient either sitting or lying down. Use a small diameter (0.7 mm and 22 G), preferably not traumatic needle. A small-gauge needle will make a smaller

hole in the Dura mater, aiding healing. Usage of a non-traumatic needle will reduce the chance of blood contamination in the CSF.

Each laboratory should use one kind of polypropylene tubes only. Glass or polystyrene tubes should in no circumstances be used. Tubes of the smallest volume should be used, and these should be filled to at least 50% of their volume. It is important to have carefully recorded and validated details concerning each stored sample so that any investigator when using these samples has a precise history of the sample.

Centrifugation is only required for visually hemorrhagic samples. Centrifuge soon with recommended 2000 x g at RT for 10 min.

**Specimen storage**

It is recommended to freeze samples and store at -80°C for long time storage. It is recommended to limit the number of freeze /thaw cycles to a maximum of 1-2. Samples should be stored no longer than 2 years.

Note
For dilution of CSF use polypropylene tubes or dilute directly onto immune strips D1.

## 8.2 Ready to use components

- Allow negative control **D4**, assay buffer **D6**, staining solution **D9** and stop solution **D10** to reach RT and mix by vortex before use.

## 8.3 Reconstitution of reagents

- 1X wash solution should be prepared before the first wash step.
- Standards D3.1 – D3.6 and controls D7 and D8 should be reconstituted before starting the test.
- 1X Blocking solution for sample dilution should be prepared before starting the test.
- 1X HRP conjugate should be prepared before starting the second incubation.

## 8.4 Specimen preparation and dilution

- Allow samples to reach RT before use.
- Mix samples before use by vortexing for 6-10 s.
- For dilution of specimen use of known and pre-tested polypropylene tubes only is recommended.
  Alternatively, specimen can be diluted directly onto immune strips by transfer of D6 followed by transfer of specimen for well.
- Samples showing an OD higher than OD of highest standard D3.1 should be diluted more than 1:50 (CSF) respectively more than 1:10 (plasma/ serum).
- Samples showing an OD lower than OD of lowest standard D3.6 should be diluted less than 1:10.

### Dilution of CSF specimen

- For appropriate measurement of human prion protein concentration in CSF dilute specimen using assay buffer **D6** in a ratio of **1:10 to 1:50** before starting the test (see table).

| Dilution ratio | Volume of D6 | Volume of CSF specimen |
|---|---|---|
| 1:10 | 90 µl | 10 µl |
| 1:50 | 98 µl | 2 µl |

## Dilution of plasma/serum specimen

- For appropriate measurement of human prion protein concentration in plasma/ serum dilute specimen using **1X Blocking solution** in a ratio of **1:10** before starting the test (see table).

| Dilution ratio | Volume of<br>1X Blocking solution | Volume of<br>plasma/ serum specimen |
|---|---|---|
| 1:10 | 90 µl | 10 µl |

# 9 Immunoassay procedure

1. Transfer 100 µl of each pre-diluted **sample** from polypropylene tube onto immune strips. For dilution of samples directly in the plate pipet assay buffer **D6** (for CSF) or **1X Blocking solution** (for plasma/ serum) followed by samples and mix 3-5 x using pipet. Pipetting of duplicates of each sample is recommended.

2. Transfer 100 µl of each reconstituted standards **D3.1-D3.6**, controls **D7** and **D8** and blank **D4** onto immune strips. Pipetting of duplicates is recommended.

NOTE
Avoid contamination of reagents, pipettes and wells/tubes by using different disposables between different samples and components. Do not interchange caps. Do not re-use any well, tube or reagent.

3. Cover the strips with sealing tape and incubate at 37°C for 60 min.

4. Remove cover and wash **5** times with 300 µl Wash buffer manually or by use of a plate washer.

NOTE
Pull off the sealing tape carefully to avoid cross-contamination.

5. Transfer 100 µl of 1:20 diluted HRP conjugate **D5** into each well.

6. Cover the strips with sealing tape and incubate at RT for 60 min.

7. Remove cover and wash **5** times with 300 µl Wash buffer manually or by use of a plate washer.

NOTE
Staining should be performed immediately after washing step 7 within 5 min.

8. Pipette 100 µl of **staining solution D9** into each well.

9. Incubate plate at RT in the dark for 15 min.

10. Stop the substrate reaction by adding 150 µl of **stop solution D10** into each well.

11. Measurement of absorbance: Mix plate with shaker of the reader for 3-5 s and let it settle down for 5 s. Measure the OD at 450 nm using 620 nm as reference wave length within 10 minutes after termination of the reaction.

Note
In high concentrated controls or samples staining components may be precipitated some time after termination. In this case additional mixing before reading is recommended.

# 10 Data analysis

The OD of the measured values is determined as the difference of the measured OD at 450 nm minus the OD at reference wavelength 620 nm ($OD_{450/620\ nm}$).

## 10.1 Quality criteria of the assay

- $OD_{450/620\ nm}$ value of negative control **D4** (blank) should be **< 0.2**.
- $OD_{450/620\ nm}$ values of positive controls **D7** and **D8** should be inside range corresponding to batch specific certificate.
- $R^2$ of the calibration curve should be ≥ 0.99.

## 10.2 Calculation of unknown prion protein concentration

For the determination of the prion protein concentration in controls and samples the automatic data analysis by means of reader software, usually the logistic regression with 4 or 5 parameters or logit-log method is recommended. The standard curve typically shows a linear progression between the plateau of the highest standard **D3.1** (20 ng/ml) and the lowest standard **D3.6** (1 ng/ml).

Note
Dilution factor must be included for estimation of real concentration of prion protein within samples after exponentiation.

Note
Samples with a measured OD smaller than the OD of the lowest standard D3.6 can be reported in terms of prion protein concentration <1 ng/ml.

# 11 References

**Breitling LP, et al. 2012.** Association of prion protein with cognitive functioning in humans. *Exp Gerontol.* 2012, S. 47(12):919-24.

**Dorey A, et al. 2015.** Association of cerebrospinal fluid prion protein levels and the distinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA Neurol.* 2015, S. 72(3):267-75.

**Vallabh, Sonia M., et al. 2019.** Prion protein quantification in human cerebrospinal fluid as a tool for prion disease drug development. *Proc Natl Acad Sci USA.* 2019, S. 116(16):7793-7798.

**Vanderstichele H, et al. 2012.** Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement.* 2012, S. 8(1):65-73.

# 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 |
| | 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.<br>Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.<br>Voir MATERIEL FOURNI pour les symbôles des composants du kit.<br>Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.<br>Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.<br>Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.<br>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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