DRG® HNP 1-3 (human) (EIA-5139)

RUO

As of 25 Feb. 2010 rm (Vers. 1.0)

Please use only the valid version of the package insert provided with the kit.

ATTENTION

For use in laboratory research only. Not for clinical or diagnostic use

Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the certificate of quality control for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. DRG is not responsible for any patent infringements that might result from the use or derivation of this product.

1 INTENDED USE

The human HNP 1-3 (Neutrophil Defensins) ELISA kit is to be used for the in vitro quantitative determination of human HNP 1-3 in plasma and cell culture supernatant samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures. The analysis should be performed by trained laboratory professionals.

2 INTRODUCTION

Human neutrophil defensins (alpha-defensins, HNP 1-3) belong to the family of cationic trisulfide-containing microbicidal peptides. Besides microbicidal, the peptides exert chemotactic, immunomodulating and cytotoxic activity and participate in host defense and inflammation. Azurophilic granules of neutrophils contain human Neutrophil Peptide (HNP)-1-4 which are highly homologous. The three principal human defensins, HNP 1-3, are unique to neutrophils and account for about 99 percent of the total defensin content of these cells. Measured amount of defensins is 3-5 mg per million human neutrophils.

Activation of neutrophils leads to rapid release of defensins. Thus, only one cell type, neutrophils, may be the source of HNP 1-3 measured in plasma and other body fluids during infection and inflammation. In normal plasma low levels of HNP 1-3 are present ranging from undetectable level to 50-100 ng/ml, while in septic conditions the levels of HNP 1-3 might be elevated to 10 mg/ml and even more. Activation of neutrophils in blood as occurs during clotting, as well as long storage of anticoagulated blood leads to a release of HNP, thus careful plasma sampling is important for possible detection of HNP. Defensins are relatively resistant to proteolysis, low pH and boiling, but have a tendency to bind to a variety of materials, including plastic and proteins.

3 KIT FEATURES

Working time of 3½ hours.
Minimum concentration which can be measured is 41 pg/ml.
Measurable concentration range of 41 to 10,000 pg/ml.
Working volume of 100 µl/well.

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Cross-reactivity

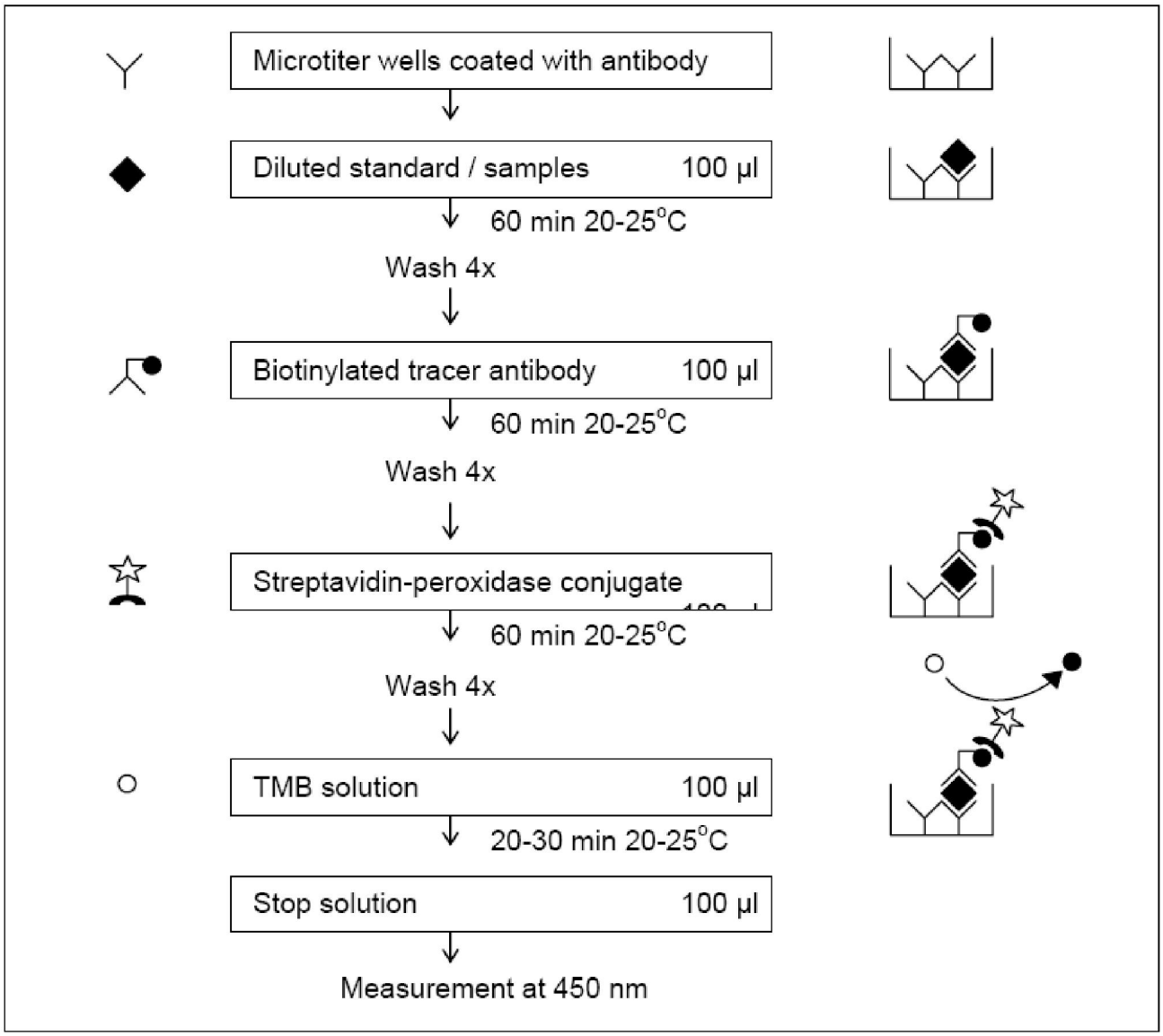
Potential cross-reacting proteins detected in the human HNP 1-3 ELISA:

Cross reactant	Reactivity
Rhesus monkey	Strong

Table 1

Cross-reactivity for other species or proteins/peptides has not been tested.

4 PROTOCOL OVERVIEW



- The human HNP 1-3 ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
- The efficient format of 2 plates with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in micro titer wells coated with antibodies recognizing human HNP 1-3.
- Biotinylated tracer antibody will bind to captured human HNP 1-3.

- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
- Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human HNP 1-3 standards (log).
- The human HNP 1-3 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

5 KIT COMPONENTS AND STORAGE INSTRUCTIONS

Item no.	Kit component	Quantity	Color code
Vial 1	Wash buffer 40x	1 vial (20 ml)	Grey
Vial 2A	Dilution buffer 10x	1 vial (20 ml)	Gold
Vial 2B	Plasma diluent 10x	1 vial (10 ml)	Gold
Vial 3	Standard	1 vial, 1 ml lyophilized	Yellow
Vial 4	Tracer, biotinylated	2 vials, 1 ml lyophilized	Green
Vial 5	Streptavidin-peroxidase	1 vial, 1 ml lyophilized	Blue
Vial 6	TMB substrate	1 vial (20 ml)	Purple
Vial 7	Stop solution	1 vial (20 ml)	Red
Item 8	12 Microtiter strips, pre-coated	2 plates	
Item 9	Frame	1	
Item 10	Adhesive covers	4	
Item 11	Certificate of quality control	1	
Item 12	Manual	1	
Item 13	Data collection sheet	1	

Table 2

Upon receipt, store individual components at 2 - 8°C. Do not freeze.
Do not use components beyond the expiration date printed on the kit label.
The standard, tracer and streptavidin-peroxidase are stable in lyophilized form until the expiration date indicated on the kit label, if stored at 2 - 8°C.
The exact concentration of the standard is indicated on the label of the vial and the certificate of quality control.
Once reconstituted, standard, tracer and streptavidin-peroxidase are stable for 1 month if stored at 2 - 8°C.
Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured.
Any irregularities to aforementioned conditions may influence plate performance in the assay.
Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed until expiration date if stored at 2 - 8°C.

5.1 Materials required but not provided

Calibrated micropipettes and disposable tips.
Distilled or de-ionized water.
Plate washer: automatic or manual.
In case a plate washer is used the supplied wash buffer is not sufficient. Additional wash buffer can be ordered separately. Please contact your local distributor.
Polypropylene tubes.
Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.

6 WARNINGS AND PRECAUTIONS

For research use only, not for diagnostic or therapeutic use.
This kit should only be used by qualified laboratory staff.
Do not add under any circumstances sodium azide as preservative to any of the components.
Do not use kit components beyond the expiration date.
Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
The assay has been optimized for the indicated standard range. Do not change the standard range.
Standard, tracer and streptavidin-peroxidase vials should be opened after reconstitution. Open vials carefully: vials are under vacuum.
Do not ingest any of the kit components.
Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advise immediately.
The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advise.
Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
Do not reuse micro wells or pour reagents back into their bottles once dispensed.
Handle all biological samples as potentially hazardous and capable of transmitting diseases.
Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.

7 SAMPLE PREPARATION

7.1 Collection and handling

Plasma

Please be aware that human HNP 1-3 is released from neutrophils into serum in the process of blood coagulation. This will lead to false positive results. It is therefore advised to use 'careful plasma', which can be obtained as follows.

Keep freshly collected blood on ice. Within 20 minutes after blood sampling, separate plasma by centrifugation: 1500xg at 4°C for 15 min. Remove plasma and transfer to fresh polypropylene tube. Be careful to not disturb white cells in the buffy coat. Recentrifuge the transferred plasma in order to avoid every contamination with white blood cells (1500xg at 4°C for 15 min).

Note that most reliable results are obtained with heparin or EDTA plasma.

7.2 Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human HNP 1-3. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human HNP 1-3 activity and give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

7.3 Dilution procedures

HNP 1-3 are highly absorbing to Ig, other proteins and plastics. Samples can only be measured accurately if diluted with supplied dilution buffer.

Plasma samples

Human HNP 1-3 can be measured accurately if plasma samples are diluted at least 2000x with supplied plasma dilution buffer in polypropylene tubes.

Rhesus monkey plasma samples need to be diluted at least 4x for accurate measurement.

Note that most reliable results are obtained with heparin or EDTA plasma.

Remark regarding recommended sample dilution

The recommend dilution for samples should be used as a guideline. The recovery of human HNP 1-3 from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of human HNP 1-3.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

8 REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Wash buffer

Prepare wash buffer by mixing 20 ml of 40x wash buffer with 780 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 40x wash buffer with 39 parts of distilled or deionized water.

Dilution buffer

Prepare dilution buffer by mixing 20 ml of the 10x dilution buffer with 180 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.

Standard/sample dilution buffer

Prepare standard/sample dilution buffer by mixing 10 ml of the 10x plasma diluent with 90 ml of prepared dilution buffer, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of standard/sample dilution buffer by diluting 1 part of the 10x plasma diluent with 9 parts of dilution buffer.

Standard solution

The standard is reconstituted by injection of 1 ml of distilled or de-ionized water. Prepare each human HNP 1-3 standard in polypropylene tubes by serial dilution of the reconstituted standard with standard/sample buffer as shown in Table 3.

Tube	Volume standard/sample buffer	Volume standard	Concentration (pg/ml)
1	See certificate of quality control	150 µl vial 3	10000
2	225 µl	150 µl tube 1	4000
3	225 µl	150 µl tube 2	1600
4	225 µl	150 µl tube 3	640
5	225 µl	150 µl tube 4	256
6	225 µl	150 µl tube 5	103
7	225 µl	150 µl tube 6	41
8	225 µl	-	0

Table 3

Tracer solution

The tracer is reconstituted by injection of 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml tracer with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of tracer by diluting 1 part of the reconstituted tracer with 11 parts of dilution buffer.

Streptavidin-peroxidase solution

The streptavidin-peroxidase is reconstituted by injection of 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml streptavidin-peroxidase with 23 ml dilution buffer, which is sufficient for 2 x 96 tests. In case less volume is desired, prepare the required volume of streptavidin-peroxidase solution by diluting 1 part of the reconstituted streptavidin-peroxidase with 23 parts of dilution buffer.

9 ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary micro well strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells.
3. Apply an adhesive cover to the tray. Tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at room temperature.
5. Wash the plates 4 times with wash buffer using a plate washer or as follows:
   a. Carefully remove the plate sealer, avoid splashing.
   b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
   c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
   d. Repeat the washing procedure 5b/5c three times.
   e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µl of diluted tracer to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray with an adhesive cover. Incubate the tray for 1 hour at room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of diluted streptavidin-peroxidase to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray with an adhesive cover, incubate the tray for 1 hour at room temperature.
11. Repeat the wash procedure described in step 5.
12. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
13. Cover the tray with a new adhesive cover, incubate the tray for 20 – 30 minutes at room temperature. Avoid exposing the micro well strips to direct sunlight. Covering the plate with aluminium foil is recommended.

14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
15. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

10 INTERPRETATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, control and samples.

If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.

The mean absorbance of the zero standard should be less than 0.3.

Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale). For an example of the standard curve see certificate of quality control included with the kit. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.

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

Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

11 TECHNICAL HINTS

User should be trained and familiar with ELISA assays and test procedure.

If you are not familiar with the ELISA technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.

Since exact conditions may vary from assay to assay, a standard curve must be established for every run. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.

Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.

Each time the kit is used, fresh dilutions of standard, sample, tracer, streptavidin peroxidase and buffers should be made.

Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.

To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

To ensure accurate results, proper adhesion of supplied covers during incubation steps is necessary.

The waste disposal should be performed according to your laboratory regulations.

12 QUALITY CONTROL

The certificate of quality control included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the certificate of quality control are to be used as a guideline only. The results obtained by your laboratory may differ.

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This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the DRG immunoassay, the possibility of interference cannot be excluded.

For optimal performance of this kit, it is advised to work according to good laboratory practice.

13 TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data shall be sent to drg@drg-diagnostics.de. Suggestions summarized below in Table 4 can be used as guideline in case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•		Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•		•			Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
		•			Especially in case of 37°C incubation: plates are not incubated uniformly
•					Assay performed before reagents were adapted to room temperature
•	•	•	•	•	Procedure not followed correctly
				•	Omission of a reagent or a step
		•			Poor mixing of samples
	•		•		Low purity of water
	•	•			Strips were kept dry for too long during/after washing
	•	•	•		Inefficient washing
	•	•			Cross-contamination from other samples or positive control
		•	•		TMB solution is not clear or colorless
•	•				Wrong filter in the micro titer reader
	•	•			Air bubbles

		•			Imprecise sealing of the plate after use
•					Wrong storage conditions

Table 4

14 REFERENCES / LITERATURE

1. Zhang, L et al; Contribution of human alpha-defensin-1, -2 and -3 to the anti-HIV-1 activity of CD8 antiviral factor. Science 2002, 298: 995
2. Chang, T et al; CAF-mediated human immunodeficiency virus (HIV) type 1 transcriptional inhibition is distinct from alpha-defensin-1 HIV inhibition. J Virol 2003, 77:6777
3. Espinoza, J et al; Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. J Matern Fetal Neonatal Med 2003, 13: 2
4. Nelsestuen, G et al; Proteomic identification of human neutrophil alpha-defensins in chronic lung allograft rejection. Proteomics 2005, 5: 1705
5. Baroncelli, S et al; Characterization of α-defensins plasma levels in Macaca fascicularis and correlations with virological parameters during SHIV89.6Pcy11 experimental infection. AIDS Res Hum Retroviruses 2007, 23: 287
6. Agratti, C et al; Activated Vγ9Vδ2 T cells trigger granulocyte functions via MCP-2 release. J Immunol. 2009, 182: 522

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..