Product information

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PTH intact ELISA

CE

IVD

REF DE3645

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Please use only the valid version of the Instructions for Use provided with the kit.

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1 INTENDED USE

The PTH intact ELISA is intended for in vitro diagnostic use in the quantitative determination of Intact PTH (Parathyroid Hormone) in human serum. This assay is intended to be used to detect elevated or decreased PTH level in human serum, and to aid in the diagnosis of hyperparathyroidism and hypoparathyroidism for professional use.

2 SUMMARY AND EXPLANATION

PTH (Parathyroid hormone, Parathormone, Parathyrin) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone, a larger molecular precursor consisting of 115 amino acids. Following sequential intracellular cleavage of a 25-amino acid sequence, preproparathyroid hormone is converted to an intermediate, a 90-amino acid polypetide, proparathyroid hormone. With additional proteolytic modification, proparathyroid hormone is then converted to parathyroid hormone, an 84 amino acid polypeptide. In healthy individuals, regulation of parathyroid hormone secretion normally occurs via a negative feedback action of serum calcium on the parathyroid glands. Intact PTH is biologically active and clears very rapidly from the circulation with a half-life of less than four minutes[1]. PTH undergoes proteolysis in the parathyroid glands, but mostly peripherally, particularly in the liver but also in the kidneys and bone, to give N-terminal fragments and longer lived C-terminal and midregion fragments. In subjects with renal insufficiency, C-terminal and midregion PTH assays typically give elevated PTH results, as reflected by impaired renal clearance[2].

3 CLINICAL SIGNIFICANCE

Intact PTH assays are important for the differentiation of primary hyperparathyroidism from other (non-parathyroid-mediated) forms of hypercalcemia, such as malignancy, sarcoidosis and thyrotoxicosis[2]. The measurement of parathyroid hormone is the most specific way of making the diagnosis of primary hyperparathyroidism. In the presence of hypercalcemia, an elevated level of parathyroid hormone virtually establishes the diagnosis. In over 90% of patients with primary hyperparathyroidism, the parathyroid hormone will be elevated[3]. The most common other cause of hypercalcemia, namely hypercalcemia of malignancy, is associated with suppressed levels of parathyroid hormone[3] or PTH levels within the normal range[4]. When Intact PTH level is plotted against serum calcium, the Intact PTH concentration for patients with hypercalcemia of malignancy is almost always found to be inappropriately low when interpreted in view of the elevated serum calcium[3,4,5]. Unlike C-terminal and midregion PTH, which typically are grossly elevated in subjects with renal insufficiency, Intact PTH assays are less influenced by the declining renal function[5]. PTH values are typically undetectable in hypocalcemia due to total hypoparathyroidism, but are found within the normal range in hypocalcemia due to partial loss or inhibition of parathyroid function.

4 PRINCIPLE OF THE TEST

The Demeditec PTH Intact Immunoassay is a two-site ELISA [Enzyme-Linked Immuno-Sorbent Assay] for the measurement of the biologically Intact 84 amino acid chain of PTH. Two different goat polyclonal antibodies (capture and detection antibody) to human PTH have been purified by affinity chromatography to be specific for well-defined regions on the PTH molecule. The biotinylated capture antibody binds only the mid-region and C-terminal PTH 39-84. The horseradish peroxidase [HRP]-labeled detection antibody binds only the N-terminal PTH 1-34.

Streptavidin Well - Biotinylated Anti-PTH (39-84) --Intact PTH -- HRP conjugated Anti-PTH (1-34)

The Intact PTH 1-84 forms the sandwich complex necessary for detection by binding with both the biotinylated capture antibody and HRP labelled detection antibody. The capacity of the biotinylated antibody and the streptavidin coated microwell both have been adjusted to exhibit negligible interference by inactive fragments, even at very elevated levels.
In this assay, calibrators, controls, or patient samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled antibody in a streptavidin-coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components. After washing, the plate is incubated with the substrate, tetramethylbenzidine (TMB). In the presence of HRP enzyme, TMB substrate is converted to a blue color. After the substrate incubation, an acidic stopping solution is added to stop the reaction and convert the color to yellow. The intensity of the yellow color is directly proportional to the concentration of Intact PTH in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of Intact PTH present in the controls and patient samples are determined directly from this curve.

5 KIT COMPONENTS

Kit Components	Description	Quantity
BIOTIN Ab Reagent 1	Biotinylated PTH Antibody	1 x 7.0 mL
PEROX Ab Reagent 2	Peroxidase (Enzyme) labeled PTH Antibody	1 x 7.0 mL
SUB TMB Reagent B	TMB Substrate [tetramethylbenzidine]	1 x 20 mL
SAM DIL Reagent 3	Diluent [equine serum] for Patient Samples read off-scale	1 x 2 mL
WASH SOLN 20x Reagent A	ELISA Wash Concentrate [Saline with surfactant]	1 x 30 mL
STOP SOLN Stopping Solution	ELISA Stop Solution [1 N sulfuric acid]	1 x 20 mL
REC SOLN Reagent 4	Reconstitution Solution containing surfactant	1 x 5 mL
SORB MT Microplates	One holder with Streptavidin Coated Strips.	12 x 8-well strips
CAL A – F LYO Calibrators A: 0 pg/mL; B – F: Refer to QC data sheet for exact concentrations	Lyophilized synthetic h-PTH. Lyophilized Zero calibrator [BSA solution with goat serum]. All other calibrators consist of synthetic h-PTH (1-84) in BSA solution with goat serum.	1 x 0.5 mL per level
CONTROL 1 & 2 LYO Controls 1 & 2: Refer to QC data sheet for exact concentrations	Lyophilized. 2 Levels. Synthetic h-PTH (1-84) in BSA solution with goat serum.	1 x 0.5 mL per level

5.1 Material and Equipment required but not provided

- Microplate reader.
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Precision Pipettors to deliver 25, 100 and 150 µL.
- (Optional): A multi-channel dispenser or a repeating dispenser for 50, 100 and 150 µL.
- Microplate Shakers: Demeditec has found for shaker diameters indicated below, the Streptavidin kits will maintain optimal performance response at the following speed settings:

Microplate Shakers	Shaking diameter	Speed setting
Orbital	3 mm (0.1118 in)	600 ± 10 rpm
	19 mm (0.75 in)	170 ± 10 rpm
Linear	25 mm (0.98 in)	170 ± 10 rpm

6 WARNINGS AND PRECAUTIONS FOR USERS

Material safety data sheets (MSDS) are available upon request.
CAUTION POTENTIAL BIOHAZARD
Although the reagents provided in this kit has been specifically designed to contain no human blood components, the human patient samples, which might be positive for HBsAg, HBcAg or HIV antibodies, must be treated as potentially infectious biohazard. Common precautions in handling should be exercised, as applied to any untested patient sample.
CAUTION
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
Stopping Solution consists of 1 N Sulfuric Acid. This is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. Use only in well-ventilated areas. If turbidity is observed in any reagent, do not perform assay and please contact your supplier.

7 SAMPLE COLLECTION AND STORAGE

The determination of Intact PTH should be performed with human serum.
To assay the specimen in duplicate, 50 µL of serum is required.
Collect whole blood without anticoagulant. After allowing blood to clot, the serum should be promptly separated, preferably in a refrigerated centrifuge and tested as soon as possible.
If serum samples cannot be run immediately, stored it at -20°C or lower.
Serum samples frozen at -20°C are stable for up to 4 months.

8 REAGENT PREPARATION AND STORAGE

Store all kit components at 2 °C - 8 °C

1. All reagents except the calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2 °C - 8 °C.
2. For each of the **calibrators** (Calibrator A through F) and **kit controls** 1 and 2, reconstitute each vial with 500 µL of Reagent 4 (Reconstitution Solution) and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. **Use the calibrators and controls as soon as possible upon reconstitution. Freeze (-20°C) the remaining calibrators and controls as soon as possible after use.**
   Standards and controls are stable at -20 °C for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in ”Procedural Notes” section.
3. Reagent A: **Wash Concentrate**; Mix contents of wash concentrate thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 4 °C, dissolve by placing the vial in a 37 °C water bath or oven with swirling or stirring. Add wash concentrate (30 mL) to 570 mL of distilled or deionized water and mix.
   The diluted working wash solution is stable for 90 days when stored at room temperature.

9 ASSAY PROCEDURE

1. Place sufficient **Streptavidin Coated Strips** in a holder to run all six (6) PTH calibrators, A - F of the Intact PTH CALIBRATORS [Exact concentration is stated on the QC data sheet], Quality Control Sera and patient samples.
   At a minimum, designate two wells to serve as “blanks”. Refer to Step 9 for final plate reading.
2. Pipet **25 µL** of calibrators, controls, and samples into the designated or mapped well.
   Freeze (-20 °C) the remaining calibrators and controls as soon as possible after use.
3. Add or dispense **50 µL** of Reagent 1 (Biotinylated Antibody) into each of the wells which already contain the calibrators, controls, and samples.
4. Add or dispense **50 µL** of Reagent 2 (Enzyme Labeled Antibody) into each of the same wells. Cover the microplate(s) with aluminium foil or a tray to avoid exposure to light, and place it on a **shaker** set at recommended settings (see section 5.1)for **3 hours** ± 30 minutes at room temperature (22 °C - 28 °C).
5. First aspirate the fluid completely and then wash/aspirate each well five (5) times with the Working Wash Solution (prepared from Reagent A), using an automatic microplate washer. The wash solution volume should be set to dispense 0.35 mL into each well.
6. Add or dispense **150 µL** of the Reagent B (TMB Substrate) into each of the wells, except the blank wells.
7. With appropriate cover to avoid light exposure, place the microplate(s) on a **shaker** set at recommended settings (see section 5.1) for **30 ± 5 minutes** at room temperature (22 °C - 28 °C).
8. Add or dispense **100 µL** of the Stopping Solution into each of the wells, except the blank wells. Mix gently.

9. Prior to reading, ensure both “blank wells” as mentioned in Step 1 are filled with 250 µL of distilled or deionized water. Blank the plate reader according to the manufacturer’s instructions by using the blank wells.*
   Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm**.
   Read the plate **again** with the reader set to **405 nm** also against distilled or deionized water.

   * If due to technical reasons the ELISA plate reader cannot be adjusted to zero using “blank,” subtract the “blank,” absorbance value from all other absorbance values to obtain results.

   Note: The second reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest calibrator, which is approximately 700 – 1,000 pg/mL. Hence, patient samples with PTH > 200 pg/mL can be quantified against a calibration curve consisting of the readings all the way up to the concentration equivalent to the highest calibrator using the 405 nm reading, away from the wavelength of maximum absorbance. In general, patient and control samples should be read using the 450 nm for PTH concentrations up to 200 pg/mL. PTH concentrations above 200 pg/mL should be interpolated using the 405 nm reading.
10. By using the final absorbance values obtained in the previous step, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the Intact PTH.

9.1 Procedural Notes

- Intact PTH 1-84 is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all calibrators, controls, and patient samples.
- It is recommended that all calibrators, controls, and patient samples are assayed in duplicate. The average absorbance units of duplicate sets should then be used for reduction of data and the calculation of results.
- The samples should be pipetted into the well with minimum amount of air-bubble. To achieve this, ”reverse pipet” described in the package insert of the manufacturers of Pipettors is recommended.
- Patient samples with values greater than the highest calibrator (Calibrator F), which is approximately 700 - 1,000 pg/mL (see exact concentration on QC data sheet), may be diluted with Reagent 3 (Sample Diluent) and reassayed. Multiply the result by the dilution factor.
- Reagents from different lot numbers must not be interchanged.
- If preferred, mix in equal volumes, in sufficient quantities for the assay, Reagent 1 (Biotinylated Antibody) and Reagent 2 (Enzyme Labeled Antibody) in a clean amber bottle, then use 100 µL of the mixed antibody into each well. This alternative method should replace Step (3) and (4), to be followed with the incubation with orbital shaker.

10 CALCULATION OF RESULTS

Curve fitting Method: Computer programs using cubic spline or 4 PL [4 Parameter Logistics] can generally give a good fit.

Important Note: if the OD 450 nm of Calibrator A after blanking is ≥ 0.100, the curve is invalid and no patient results should be reported.

Sample Data *at 450 nm* [raw A.U. readout against distilled or deionized water]

Microplate Well	**1st Reading** Absorbance Unit	**2nd Reading** Absorbance Unit	**Average** Absorbance Unit	**Intact PTH pg/mL**	**Intact PTH pg/mL –Result to report**
Calibrator A	0.020	0.016	0.018		0
Calibrator B	0.056	0.051	0.054		7
Calibrator C	0.124	0.119	0.122		18
Calibrator D	0.388	0.393	0.391		55
Calibrator E	1.335	1.340	1.338		210
Control 1	0.200	0.200	0.200	27.6	27.6
Control 2	0.804	0.794	0.799	119	119
Patient Sample 1	0.147	0.136	0.142	19.1	19.1
Patient Sample 2	0.407	0.409	0.408	58.5	58.5
Patient Sample 3	2.375	2.454	2.415	> 200	*
Patient Sample 4	3.725	3.725	3.725	> 200	*

* Because the concentration readout is > 200 pg/mL, it is recommended to use the data obtained at 405 nm as shown in **Sample Data** *at 405 nm* in the table below.

Sample Data *at 405 nm* [raw A.U. readout against distilled or deionized water]

Microplate Well	**1st Reading** Absorbance Unit	**2nd Reading** Absorbance Unit	**Average** Absorbance Unit	Intact PTH **pg/mL**	**Intact PTH pg/mL – Result to report**
Calibrator A	0.014	0.008	0.011		0
Calibrator D	0.124	0.128	0.126		55
Calibrator E	0.428	0.425	0.427		210
Calibrator F	1.309	1.317	1.313		700
Control 1	0.074	0.066	0.070	< 200	DNR*
Control 2	0.260	0.251	0.256	121	DNR*
Patient Sample 1	0.049	0.043	0.046	< 200	DNR*
Patient Sample 2	0.132	0.133	0.133	< 200	DNR*
Patient Sample 3	0.758	0.782	0.770	401	401
Patient Sample 4	1.314	1.321	1.318	> 700	DNR*

¶ ***DNR:** For samples with readout < 200 pg/mL, it is recommended to use the data obtained at 450 nm as shown in **Sample Data** *at 450 nm* in the table above. This practice should give the results with optimum sensitivity of the assay.
Although the readout for Control (2) < 200 pg/mL**,** it is recommended that the actual result be read out and recorded for quality control evaluation purposes. Further, absorbance for Control 2 is sufficiently high to be analytically valid.
If the absorbance readout is off-scale or higher than the average absorbance of the highest calibrator. Sample should be repeated with dilution.

*NOTE: The data presented are **for illustration purposes only** and must not be used in place of data generated at the time of the assay.*

11 QUALITY CONTROL

Two distinct levels of Intact PTH controls (Control 1 & 2) with an established control range are provided in each kit for quality control purposes. In addition, other suitable control material established by each laboratory can be used. PTH Controls should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the PTH control (Control 1 or 2) values lie outside the acceptable control range, the results for the patient sample should be considered invalid. If the OD 450 nm of Calibrator A after blanking is ≥ 0.100, the curve is invalid and no patient results should be reported.

12 LIMITATIONS OF PROCEDURE

The PTH intact ELISA kit has exhibited no “high dose hook effect” with samples spiked with 2,100,000 pg/mL of Intact PTH. Samples with Intact PTH levels greater than the highest calibrator, however, should be diluted and reassayed for correct values. Like any analyte used as a diagnostic adjunct, Intact PTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests. Because of the relationship between PTH and calcium in various disorders, PTH results should be interpreted in the context of serum calcium and the patient’s clinical history. The PTH intact ELISA will detect non-Intact PTH (1-84) such as PTH fragment (7-84). PTH fragment (7-84) may cause falsely elevated Intact PTH results in patients with abnormal renal function because these patients may have various concentrations of PTH fragment (7-84) in their blood. For patients with abnormal renal function please interpret the Intact PTH results with caution and do not make patient management decisions on the Intact PTH result alone. The PTH intact ELISA assay is unaffected by the following interference substances: Triglycerides (<15 mg/mL), bilirubin (<0.2mg/mL) and hemoglobin (<5.0mg/mL). Supplements containing high biotin levels such as those marketed for hair, skin, and nail benefits, may contain interfering biotin amounts. Biotin levels higher than the recommended daily allowance may cause interference with the assay. Therefore, it is important to communicate with health care providers and patients about biotin intake when collecting samples to prevent incorrect test results. Results show that the highest concentration at which no significant interference was observed is 5 ng/mL of D-Biotin. Samples from patients routinely exposed to animals or animal serum products may contain heterophilic antibodies that react with the reagent antibodies, potentially causing falsely elevated results. This assay has been formulated to mitigate the risk of this type of interference. However, potential interactions between patient sera and test components can occur. The use of full or semi-automated equipment for dispensing of reagents and/or washing of the plate must be validated for equivalency to manual results by the laboratory. For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

13 EXPECTED VALUES

Intact PTH levels were measured in 28 apparently normal individuals in the U.S. with the PTH intact ELISA. The values obtained ranged from **8.8 to 76.6 pg/mL** for serum. Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution. The geometric mean ± 2 standard deviations of the mean were calculated to be **8.3 to 68.0 pg/mL** for serum. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, establish its own reference ranges.

14 PERFORMANCE CHARACTERISTICS

14.1 Traceability

The Demeditec PTH intact calibrators are traceable to the WHO international standard PTH (1-84) recombinant NIBSC 95/646.

14.2 Accuracy/Method Comparison

One hundred twenty-three patient samples, with Intact PTH values ranging from 3.2 to 805 pg/mL were assayed by the PTH intact ELISA procedure and the PTH Immunoradiometric Assay Linear regression analysis gives the following statistics. Three hundred and nine (309) patient samples, with Intact PTH values ranging from 1.0 to 833 pg/mL were assayed by the previous Demeditec PTH kit and the updated Demeditec PTH kit. Linear regression analysis gives the following statistics:

Demeditec ELISA = 0.997 IRMA Kit + 2.9 pg/mL
r = 0.990 N = 123

14.3 Detection Capability

The Limit of Blank (LoB), the Limit of Detection (LoD), and the Limit of Quantitation (LoQ) were determined for Intact PTH intact ELISA assay per CLSI EP17-A2 Guideline. The LoB is defined as the highest measurement result that is likely to be observed for a blank sample. The Intact PTH assay has a LoB of 0.2 pg/mL. The Limit of Detection limit (LOD) is defined as the lowest concentration of Intact PTH that can be detected with 95% probability. The PTH intact ELISA has a LOD of 1.8 pg/mL. The LoQ is defined as the lowest concentration of Intact PTH that can be detected at a total CV less than 20%. The Intact PTH assay has an LoQ of 11.4 pg/mL.

14.4 Specificity and Cross-Reactivity

The antibodies used in the PTH intact ELISA were purified by affinity chromatography to be specific for well-defined regions on the PTH molecule. The peroxidase labeled antibody recognizes only the N-terminal region or the 1-34 amino acid sequence of the PTH molecule; whereas the biotinylated antibody is specific to the 39-84 segment. Accordingly, only Intact PTH, which requires binding by both the enzyme conjugated and biotinylated antibodies, can be detected by this assay.
To further achieve the specificity of this assay, conjugation and biotinylation and the molar ratios thereof, have been optimized to minimize detection of fragments of PTH. Human PTH 1-34 at levels up to 300 pg/mL and the C-terminal 39-84 fragment at levels up to 300,000 pg/mL give molar cross-reactivities to PTH of less than 2% and 0.02%, respectively. Human PTH 7-84 at level up to 1,000 pg/mL showed 44.5% cross-reactivity.

14.5 Precision and Reproducibility

The precision (intra-assay variation) of the PTH intact ELISA test was calculated from replicate determinations on each of the four samples as defined below. All determinations from each sample below were from a single assay.

Intra-Assay Variation

Sample	Mean Value (pg/mL)	N	Coefficient of Variation %
A	3.6	25	3.2
B	170.9	25	1.8
C	34.9	24	3.4
D	328.1	24	1.5

The total precision (inter-assay variation) of the PTH intact ELISA test was calculated from data on two samples obtained in 21 different assays, by three technicians on two different lots of reagents, over a three-week period.

Inter-Assay Variation

Sample	Mean Value (pg/mL)	N	Coefficient of Variation %
A	32.7	21	7.7
B	132.0	21	7.0

14.6 Recovery

Various amounts of PTH 1-84 were added to three different patient sera to determine the recovery. The results are described in the following table:

Serum Sample	PTH Conc (pg/ml)	Expected Value (pg/ml)	Measured Value (pg/ml)	Recovery (%)
Sample A	65.9			
PTH Std	1320			
A + 10% PTH Std		191	197	103.0%
A + 20% PTH Std		317	330	104.2%
A + 30% PTH Std		442	448	101.3%
Sample B	102			
PTH Std	1320			
B + 10% PTH Std		224	234	104.6%
B + 20% PTH Std		346	353	102.1%
B + 30% PTH Std		467	484	103.6%
Sample C	35.6			
PTH Std	1320			
C + 10% PTH Std		164	174	106.1%
C + 20% PTH Std		292	307	105.0%
C + 30% PTH Std		421	417	99.1%

14.7 Linearity of Patient Sample Dilutions: Parallelism

The linearity of the PTH intact ELISA was evaluated over 0 to 700 pg/mL measuring interval.
Four patient serum samples were diluted with Reagent 3 (the Diluent for Patient Samples). The results were analyzed by a linear regression of Observed PTH Concentration versus Expected PTH Concentration.

Sample	Dilution	Expected	Observed	Observed (pg/mL)
A	Undiluted	-	641	641
	1:2	161	321	309
	1:4	80.5	160	138
	1:8	40.3	80.1	78.2
B	Undiluted	-	-	>700
	1:2	115	356	356
	1:4	58	178	166
	1:8	29	89.0	96.7
C	Undiluted	-	410	410
	1:2	88	205	161
	1:4	44	103	81.2
	1:8	22	51.3	49.4
D	Undiluted	-	649	649
	1:2	213	325	300
	1:4	107	162	132
	1:8	53	80.1	74.5

The resulting equation for linear regression analysis is:

Observed PTH (y) = 1.017 Expected PTH (x) -15.587 pg/mL

$R^2 = 0.995$

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SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	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
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V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta