



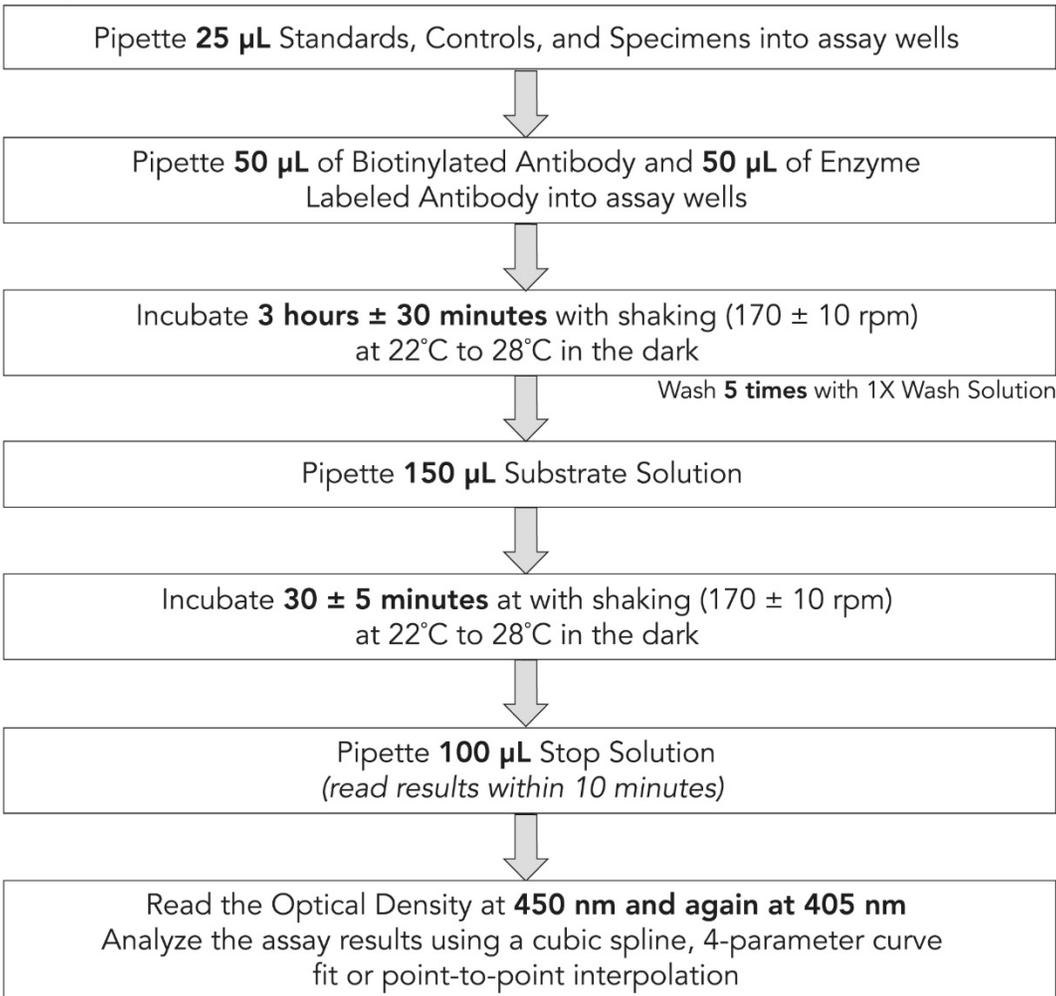
An enzyme immunoassay for the quantitation of Intact PTH (Parathyroid Hormone) in human serum

SUMMARY

Reagents, Standards, Controls, and Samples Preparation

- Dilute Wash Buffer Concentrate 1:20 with DI Water. Store at room temperature.
- Reconstitute all Standards and Controls with 500 μL Reconstitution Solution (RGT 4), 10 minutes prior to use.

Assay Procedure





INTENDED USE

The MicroVue Intact PTH EIA kit measures the amount of intact Parathyroid Hormone (PTH) in human serum.

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 polypeptide, parathyroid hormone. With additional proteolytic modification, parathyroid 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 mid-region fragments. In subjects with renal insufficiency, C-terminal and mid-region PTH assays typically give elevated PTH results, as reflected by impaired renal clearance.²

Intact PTH assays are important for the differentiation of primary hyperparathyroidism from the 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

Hypercalcemia of malignancy, is associated with suppressed levels of parathyroid hormone or PTH levels within the normal range. 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.³⁻⁵

Unlike C-terminal and mid-region 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 caused by total hypoparathyroidism, but are found within the normal range in hypocalcemia resulting from a partial loss or inhibition of parathyroid function.

PRINCIPLE OF THE PROCEDURE

The MicroVue Intact PTH EIA for the quantitation of intact PTH in human serum is a two-step procedure utilizing (1) a microassay plate coated with streptavidin and a biotinylated goat polyclonal antibody that binds specifically to human mid-region and C-terminal PTH (39-84), (2) a HRP-conjugated goat polyclonal anti-human N-terminal PTH (1-34) antibody, and (3) a chromogenic substrate.

In Step 1, Standards, Controls, and test specimens are added to microassay wells pre-coated with streptavidin. Biotin-conjugated primary polyclonal anti-human mid-region and C-terminal PTH (39-84) antibody and horseradish peroxidase (HRP)-conjugated secondary polyclonal anti-human N-terminal PTH (1-34) antibody is added to each test well. Intact PTH present in the Standards, Controls or specimens are captured in the microassay wells through binding of the biotinylated primary antibody to the streptavidin immobilized on the plate and simultaneously detected by the HRP-conjugated secondary antibody. At the end of the assay incubation, a wash cycle removes unbound material.

In Step 2, a chromogenic enzyme substrate is added to each microassay well. The bound HRP-conjugate reacts with the substrate, forming a blue color. After incubation the enzyme reaction is stopped chemically, the color changes to yellow, and the color intensity is measured spectrophotometrically at 450 nm. The color intensity of the reaction mixture is proportional to the concentration of Intact PTH present in the test specimens, Standards, and Controls.

REAGENTS AND MATERIALS PROVIDED

96 Assays for Intact PTH (Parathyroid hormone)

MicroVue Intact PTH EIA kit contains the following:

A	PTH Standards	Parts CAL A – CAL F	0.5 mL each
B	Lyophilized. Each contains purified synthetic human PTH peptide with an assigned protein concentration		
C	(pg/mL) in BSA solution with goat serum. Zero standard is BSA solution with goat serum		
D			
E			
F			
L	PTH Control 1	Part CTRL 1	0.5 mL
	Lyophilized. Contains synthetic human PTH peptide with an assigned concentration (pg/mL) in BSA solution with goat serum		
H	PTH Control 2	Part CTRL 2	0.5 mL
	Lyophilized. Contains synthetic human PTH peptide with an assigned concentration (pg/mL) in BSA solution with goat serum		
1	Microassay Plate	Part PLA	12 x 8 wells
	Eight-well strips coated with Streptavidin in a resealable foil pouch		
2	Stop Solution	Part SOLN	20 mL
	Contains 1N (3%) Sulfuric Acid (H ₂ SO ₄)		
3	20X Wash Concentrate	Part RGT A	30 mL
	Contains saline with surfactant		
4	Specimen Diluent	Part RGT 3	2 mL
	Contains equine serum		
5	TMB Substrate	Part RGT B	20 mL
	Ready to use. Contains 3,3',5,5'-tetramethylbenzidine (TMB)		
6	Biotinylated PTH Antibody	Part RGT 1	7 mL
	Contains biotin-conjugated polyclonal anti-human mid-region and C-terminal PTH (39-84) antibody		
7	Enzyme Labeled PTH Antibody	Part RGT 2	7 mL
	Contains horseradish peroxidase-conjugated polyclonal anti-human N-terminal PTH (1-34) antibody		
8	Reconstitution Solution	Part RGT 4	5 mL
	Contains saline with surfactant		

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer (60 minute range)
- Clean, unused microassay plates, 96 well dilution plate and/or test tubes and racks
- Container for wash buffer dilution
- Wash bottle or other validated immunoassay washing system
- Micropipettes and sterile, disposable pipette tips
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes
- Clean pipettes, 1 mL, 5 mL, and 10 mL
- Reagent reservoirs for adding conjugate, substrate and stop solutions to plate (use clean, unused reservoirs for each reagent)
- Plate reader capable of A_{450} and A_{405} readings between 0.0 and 4.0
- Deionized or distilled water
- Shaker/rotator: For shaker diameters indicated below, the kits will maintain optimal performance response at the following speed settings:

Microplate	Shaking diameter	Speed
Orbital	3 mm (0.1118 in)	600 ± 10 rpm
	19 mm (0.75 in)	170 ± 10 rpm
Linear	2.5 mm (0.098 in)	170 ± 10 rpm

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Intact PTH 1-84 is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all standards, controls, and patient samples.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.⁷
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Reagents from different lot numbers must not be interchanged.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- The Stop Solution is considered corrosive and can cause irritation. Do not ingest. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water. If ingested, call a physician.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure timely delivery of reagents.
- For accurate measurement of samples, add samples and Standards precisely. Pipette carefully using only calibrated equipment.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN HANDLING AND PREPARATION*).
- Avoid microbial or cross-contamination of specimens or reagents.
- Test each sample in duplicate.
- Do not use any single microassay well for more than one test.
- Using incubation times and temperatures other than those indicated in the Procedure section may give erroneous results.
- The TMB Substrate must be protected from light and contact with metal or rubber during storage and incubation. Avoid contact with eyes, skin, and clothing. If contact is made, immediately rinse affected area with water.

- Do not allow microassay wells to dry once the assay has begun.
- When removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
- To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
- A wash bottle or automated filling device should be used to wash the plate (*ASSAY PROCEDURE*, step 5). For best results, do not use a multichannel pipette to wash the microassay plate.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

Store all kit components at 2°C to 8°C except the Wash Concentrate and Stop Solution. All reagents except the Standards, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2°C to 8°C, except the Wash Concentrate and Stop Solution, which should be kept at room temperature until dilution to avoid precipitation.

REAGENT PREPARATION

Bring all reagents and materials to room temperature before use.

After removing the needed reagents and materials, return the unused items to their appropriate storage temperatures (see *STORAGE*).

Microassay Strips

Determine the number of strips needed for the assay. Quidel recommends testing the blank wells, Standards, and Controls in duplicate. Remove the unneeded strips, place them in the storage bag, reseal the bag, and return it to 2°C to 8°C. Secure the strips to be used in the assay in the assay plate frame.

Wash Solution (RGT A)

Mix the 20X Wash Solution Concentrate by inverting the bottle several times. If the 20X Wash Solution Concentrate has been stored at 2°C to 8°C, crystals may have formed. To dissolve the crystals, warm the bottle in a 37°C water bath until all crystals have dissolved, and follow by mixing thoroughly. Prepare the Wash Solution by diluting the entire contents of the bottle of 20X Wash Solution concentrate (30 mL) in 570 mL distilled or deionized water. Mix thoroughly. The diluted Wash Solution is stable for 90 days when stored in a clean container at room temperature.

Standards and Controls

For each of the Standards (CAL A through F) and kit controls 1 and 2, reconstitute each vial with 500 µL of Reconstitution Solution (RGT 4) and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Intact PTH is a very labile molecule. Use the Standards and controls immediately after reconstitution. Freeze (–20°C) the remaining Standards 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. Antibody Solutions (Optional)

If preferred, mix equal volumes of the Biotinylated Antibody (RGT 1) and Enzyme Labeled Antibody (RGT 2) in a clean, amber bottle; preparing sufficient quantity for the assay. Then, add 100 µL of the mixed antibody into each well. This alternative method will replace Step 3 and Step 4 of the Assay Procedure, followed by the incubation with the orbital shaker.

SPECIMEN HANDLING AND PREPARATION

Handle and dispose of all specimens using Universal Precautions.

Specimen Collection

CAUTION: Treat all specimens as potentially infectious. Use Universal Precautions. Do not use contaminated or improperly stored specimens.

Serum/Plasma

The determination of Intact PTH should be performed with EDTA plasma or serum. Improved PTH stability has been reported in EDTA plasma as compared with serum.⁶ The Intact PTH levels in EDTA plasma may therefore more accurately represent *in vivo* concentrations.

Serum and EDTA plasma specimens should be collected aseptically using standard techniques.⁸ After allowing blood to clot, the serum or EDTA plasma should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20°C or lower. Serum samples may be stored up to 8 hours at 2°C to 8°C . Serum samples frozen at -20°C are stable for up to 4 months.

Specimen Dilution

Specimens must be diluted so that values observed do not exceed the highest standard (CAL F), which is approximately 700-1000 pg/mL (see exact concentration on vial label). Specimens with readings outside this range should be diluted with Specimen Diluent (RGT 3) and re-assayed at the new dilution. Multiply the result by the dilution factor. Do not store or reuse diluted specimens.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

1. Place sufficient Streptavidin Coated Strips in a holder to run all six (6) of the Intact PTH Standards [A-F, exact concentration is stated on the vial label], kit controls and patient samples.
2. Pipet 25 μL of Standard, control or sample into the designated or mapped well. Freeze (-20°C) the remaining Standards and controls as soon as possible after use.
3. Add or dispense 50 μL of the Biotinylated Antibody (RGT 1) into each of the wells containing Standard, control or sample.
4. Add or dispense 50 μL of the Enzyme Labeled Antibody (RGT 2) into each of the same wells. Cover the microplate(s) with aluminum foil or a tray to avoid exposure to light. Place on an orbital shaker or rotator at 170 ± 10 rpm at room temperature (22°C to 28°C) for 3 hours \pm 30 minutes.
5. Wash the microassay wells using the following procedure:
 - a. Aspirate the contents from each well.
 - b. Using a wash bottle or automated plate washing device, add approximately 350 μL Wash Solution to each well.
 - c. Aspirate the contents from each well.
 - d. Repeat steps a-c four (4) additional times for a total of five (5) washes.
6. Add or dispense 150 μL of the TMB Substrate (RGT B) into each washed test well.
7. With appropriate cover to avoid light exposure, place the microplate (s) on an orbital shaker or rotator at 170 ± 10 rpm at room temperature (22°C to 28°C) for 30 ± 5 minutes.
8. Add or dispense 100 μL of Stop Solution to each well to stop the enzymatic reaction.
9. Gently tap the plate on the bench top to disperse the color development completely and evenly.

10. Determine the absorbance reading at 450 nm for each test well within 10 minutes after the addition of the Stop Solution (step 8), making a blank correction in accordance with the spectrophotometric system in use. Read the plate again at 405 nm, making a blank correction in accordance with the spectrophotometric system in use (blank correction: 250 μ L distilled or deionized water).
NOTE: The second reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest standard, which is approximately 700-1000 pg/mL. Hence, patient samples with PTH > 200 pg/mL can be quantified against a calibration curve consisting of the readings up to the concentration equivalent to the highest standard 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.
11. Determine the concentration of Samples and Controls from the standard curve.
12. Dispose of the remaining specimens, substrate, and the used microassay strips (see *WARNINGS AND PRECAUTIONS*).

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable, and the samples should be repeated.

INTERPRETATION OF RESULTS

Use of the Standard Curve

Using the final absorbance values obtained in Step 10 of the Assay Procedure, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the Intact PTH. Most plate reading software and computers are capable of performing these calculations. Alternatively, the data may be graphed manually. For the 450 nm readings, construct a calibration curve using the first five Standards provided, i.e. Standards A-E. For the 405 nm readings, construct a second calibration curve using the three Standards with the highest concentrations, i.e. D-F. The values (pg/mL) of the test samples can be read directly from the best-fit line of the calibration curve.

Table 1.
Sample Data at 450 nm

Sample	A ₄₅₀	pg/mL
Standard A	0.018	0
Standard B	0.054	7
Standard C	0.122	18
Standard D	0.391	55
Standard E	1.338	210
Control 1	0.200	27.6
Control 2	0.799	119
Patient Sample 1	0.142	19.1
Patient Sample 2	0.408	58.5
Patient Sample 3	2.415	*
Patient Sample 4	3.725	*

*Because the concentration readout is > 200 pg/mL, it is recommended to use the data obtained at 405 nm (see Table 2 below).

Table 2: Sample Data at 405 nm

Sample	A ₄₀₅	pg/mL
Standard A	0.011	0
Standard D	0.126	55
Standard E	0.427	210
Standard F	1.313	700
Control 1	0.070	*
Control 2	0.256	**
Patient Sample 1	0.046	*
Patient Sample 2	0.133	*
Patient Sample 3	0.770	401
Patient Sample 4	1.318	***

*For samples with readout < 200 pg/mL, it is recommended to use the data obtained at 450 nm (see Table 1 above). This practice should give the results with optimum sensitivity of the assay.

**Although the readout for Control 2 is < 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.

***The absorbance readout is off-scale or higher than the average absorbance of the highest standard. Sample should be repeated with dilution.

LIMITATIONS

The MicroVue Intact PTH EIA 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 standard, however, should be diluted and re-assayed 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.

EXPECTED VALUES

Serum from 148 apparently normal donors were tested in the MicroVue Intact PTH EIA kit. The results are presented below.

Sample	N	Mean ± 2 SD (pg/mL)
Serum	148	10.4-66.5

NOTE: The mean and Standard Deviation (SD) behavior of Intact PTH concentrations determined for serum samples may vary between laboratories. Therefore, it is recommended that each laboratory determine the mean Intact PTH concentration and standard deviation values for samples.

PERFORMANCE OF THE TEST

Limits

LOD: The limit of detection (LOD) for the Intact PTH ELISA is 1.57 pg/mL, defined as the smallest single value which can be distinguished from zero at the 95% confidence limit.

Precision

Intra-assay precision was determined by assaying 25 replicates of 2 samples.

Sample	Mean Value (pg/mL)	N	Intra-assay C.V. (%)
Sample A	32.4	25	6.08%
Sample B	178.2	25	3.68%

Inter-assay precision was determined by assaying 2 samples in 21 different assays, by three technicians on three different lots of reagents.

Sample	Mean Value (pg/mL)	N	Inter-assay C.V. (%)
Sample A	30.3	21	3.6%
Sample B	159.1	21	2.8%

Linearity

Linearity was performed by diluting serum samples with specimen diluent (RGT 3) and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
A	Undiluted	-	322	-
	1:2	161	148	92
	1:4	80.5	73.1	91
	1:8	40.3	41.5	103
B	Undiluted	-	230	-
	1:2	115	97	84
	1:4	58	55	95
	1:8	29	30	103
C	Undiluted	-	176	-
	1:2	88	82	93
	1:4	44	45	102
	1:8	22	24	109
D	Undiluted	-	426	-
	1:2	213	192	90
	1:4	107	90	84
	1:8	53	47	89

Spike Recovery

Spike Recovery was performed by spiking serum samples with a known quantity of PTH and comparing observed values with expected values.

Sample	PTH Endogenous (pg/mL)	PTH Added (pg/mL)	Expected Value (pg/mL)	Observed Value (pg/mL)	Recovery (%)
A	32.7	132	165	168	102
	20.6	264	285	288	101
	13.5	396	410	413	101
B	68.6	132	201	191	95
	51.7	264	316	344	109
	45.0	396	441	462	105
C	19.9	132	152	165	109
	15.4	264	279	275	99
	13.3	396	409	424	104

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF

8044 – MicroVue Intact PTH EIA Kit

IVD



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REF

Catalogue number



CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code



Use by



Manufacturer



Temperature limitation



Intended use



Consult e-labeling
instructions for use

IVD

For *In Vitro* diagnostic use



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control
