Calcitonin Assay

Immunoradiometric (IRMA) Assay

(Coated Tube Technology)

For the quantitative determination of Calcitonin in human serum.

For In Vitro Diagnostic use only

CE

Scantibodies

(Part Number: 3KG556)

Store at 2 - 8° C

INTENDED USE

This Scantibodies kit has been designed for the quantitative determination of Calcitonin in human serum.

PHYSIOLOGY

Calcitonin is a 32-amino-acid polypeptide that is secreted primarily by the parafollicular C-cells. Its main biological effect is to inhibit osteoclastic bone resorption. This property has led to Calcitonin's use for disorders characterized by increased resorption such as Paget's disease and for some patients with osteoporosis.
The amino acid sequences of porcine, bovine, salmon, and human Calcitonin are known, and biologically active molecules have been synthesized. The structure of each is similar, whereas the immunologic activity of Calcitonin from various species differs profoundly.

The most prominent clinical syndrome associated with a disordered hypersecretion of Calcitonin is medullary carcinoma of the thyroid (MTC). MTC is a tumor of the Calcitonin producing C-cells of the thyroid gland. MTC comprises 5 - 10% of all thyroid cancer. It may occur sporadically or in a familial form that is transmitted as an autosomal dominant trait. Despite its being a relatively uncommon tumor, MTC has great clinical importance because of its familial distribution. Further, it lent itself to be diagnosed early by serum Calcitonin. This is frequently associated with other clinical features and it has good potential for cure with surgery. Although a rare tumor, it can occur in a familial pattern as a Type II multiple endocrine neoplasias. These tumors usually produce diagnostically elevated serum concentrations of Calcitonin; therefore, the immunoassay for Calcitonin in serum can be used to diagnose the presence of MTC with an exceptional degree of accuracy and specificity. In the small but increasing percentage of patients, however, basal hormone levels are indistinguishable from normal. Some of these subjects represent the early stages of C-cell neoplasia or hyperplasia that are most amenable to surgical cure. To diagnose these patients with early disease, provocative tests for Calcitonin secretion are necessary to preclude false negatives if only basal Calcitonin determination is performed. Most tumors respond with increased Calcitonin levels to the administration of either calcium or pentagastrin or their combination, but either agent can still give misleading results. Therefore, in cases with clinical manifestations, both agents should be considered for diagnostic testing. Further, calcitonin measurements can also be used to monitor the efficacy of therapy in patients with Calcitonin producing tumors.

Neoplastic disorders of other neuroendocrine cells can also elevate Calcitonin. The best example is small cell lung cancer. Other tumors such as carcinoids and islet cell tumors of the pancreas can also result in elevated serum Calcitonin.

Increases in serum Calcitonin have also been noted in both acute and chronic renal failure, hypercalciuria and hypercalcaemia.

PRINCIPLE OF PROCEDURE

The Scantibodies Calcitonin Immunoassay is a two-site immunoradiometric assay (IRMA) for the measurement of Calcitonin. Two different goat polyclonal antibodies to human Calcitonin have been purified by affinity chromatography to be specific for well-defined regions on the Calcitonin molecule.

The sample containing Calcitonin is incubated simultaneously with a capture antibody (coated tube) and ^{125}I labeled antibody. Calcitonin present in the sample is bound by both the immobilized and labeled antibodies to form a "sandwich" complex:

Anti-Calcitonin Tube --- Intact Calcitonin (1-32) --- ^{125}I Anti-Calcitonin

At the end of the assay incubation, the tube is washed to remove unbound components. The radioactivity bound to the solid phase is measured in a gamma counter. Since the formation of a sandwich complex occurs only in the presence of a Calcitonin molecule, the radioactivity of the tube bound complex is directly proportional to the amount of Calcitonin in the sample.

A dose response curve of radioactivity vs. concentration is generated using results obtained from standards which are assayed concurrently with the unknowns. Concentrations of Calcitonin present in the controls and patient samples are determined directly from this curve.

A positive calcitonin value should be confirmed by testing the same specimen in a specific procalcitonin test in order to check for the possibility of a false positive.

REAGENTS

The Scantibodies Calcitonin Kit contains sufficient reagents for 100 single determinations. The kit is stable at 2 - 8° C until the stated expiration date.

CALCITONIN STANDARDS

One set of standards consists of six vials containing lyophilized human serum with different concentrations of human Calcitonin with 0.1% Sodium Azide. Store the vials at 2 - 8° C until the stated expiration date.

CALCITONIN CONTROL SERUM

Two vials containing lyophilized human serum with human Calcitonin and 0.1% Sodium Azide. Refer to the vial label for the lot specific control ranges. Store the vial at 2 - 8° C until the stated expiration date.

^{125}I ANTI-CALCITONIN

The tracer consists of two vials each containing 5.0 mL of ^{125}I labeled anti-Calcitonin in a buffered protein solution. Store the tracer at 2 - 8° C until the stated expiration date. Each vial contains less than 10μCi (370 kBq) of ^{125}I. This kit contains ^{125}I (half life: 60 Days), emitting ionizing X (28 keV) and Gamma (35,5 keV) radiations.

ANTI-CALCITONIN COATED TUBES

Two packages of 50 antibody coated polystyrene tubes. The tubes are coated with a goat polyclonal antibody directed against human Calcitonin. Store the tubes at 2 - 8° C protected from moisture until the stated expiration date. Allow the tubes to equilibrate to ambient temperature prior to opening package. Close the package immediately after removing the required number of tubes. Do not remove desiccants.

WASH CONCENTRATE (30x concentrated)

One vial contains 30 ml of a surfactant in phosphate buffered saline with 1.5 % Sodium Azide as a preservative.

PREPARATION OF REAGENTS

CALCITONIN STANDARDS

The Scantibodies Laboratory, Inc. Calcitonin IRMA tube kit contains the calcitonin standards prepared analytically on a mass basis from purified synthetic Calcitonin (1-32) and normal human serum. Reconstitute each vial with 2.0 mL and the zero vial with 4.0 mL of distilled or deionized water. Allow the vial to stand for 30 minutes (at room temperature), then mix thoroughly by gentle inversion to ensure complete reconstitution and place in an ice bath. Store reconstituted standards $\leq$ -20° C. Do not freeze/thaw the standards more than 3 times.

CALCITONIN CONTROL SERUM

Reconstitute each vial with 2.0 mL of distilled or deionized water. Allow the vial to stand for 30 minutes (at room temperature), then mix thoroughly by gentle inversion to ensure complete reconstitution and place in an ice bath. Store reconstituted controls $\leq$ -20° C. Do not freeze/thaw the controls more than 3 times.

WASH CONCENTRATE (30x concentrated)

Mix the contents of the wash concentrate thoroughly with 870 mL of distilled or deionized water. Store at room temperature until the stated expiration date of the kit. The wash concentrate consists of a high salt solution. Hence, a precipitate may occur at low temperatures. This precipitate, provided it is mixed thoroughly with water, has no deleterious effect.

WARNINGS AND PRECAUTIONS FOR USERS

Use of the Assay

The reagents are for In Vitro Diagnostic use only.

Human Serum Caution

The human serum in this kit has been prepared from human donors which have been tested by FDA approved immunoassays and found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Anti HIV I/II and Anti HCV. However, it is recommended to consider the standards and controls as a potential biohazard and handle them with the same precautions as applied to any untested patient sample.

Radioactivity Warning

All radioactive materials must be disposed of according to the regulations (regulations differ from country to country) and guidelines of the agencies with jurisdiction over the laboratory. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are used. Storage of radioactive materials should be limited to specifically designated and appropriately secured areas. Access to radioactive materials should be limited to authorized and trained personnel only. Do not pipette radioactive solutions by mouth. Avoid direct contact with radioactive materials by using protective articles such as lab coats and disposable gloves. Radioactive materials must be stored in designated areas in their original containers or in containers providing equivalent radiation protection. A record of disposal of all radioactive materials must be kept. Immediately remove spilled solutions and decontaminate contaminated devices. Check laboratory equipment and glassware regularly to detect contamination with radioisotopes.

This radioactive material may be received, acquired, possessed, or used only by physicians, clinical laboratories, or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the country in which the products use is intended.

Sodium Azide (NaN_3) Warning

Some reagents in the Scantibodies Calcitonin Assay contain Sodium Azide. Sodium Azide may react with

lead and copper plumbing to form highly explosive metal Azides. On disposal, flush the drain with a large volume of water to prevent Azide build-up. Avoid direct contact with skin.

SAMPLE PREPARATION AND STORAGE

The determination of human Calcitonin should be performed on serum. To assay the specimen in duplicate 400 µL of serum is required. Collect blood into a tube containing no additives. Allow the blood to clot for 1 - 4 hours (in an ice bath or refrigerator), centrifuge the tube (preferably in a refrigerated centrifuge) and then collect the serum. If the assay is not run immediately after serum collection, promptly store the sample at -20° C. Avoid grossly hemolyzed or grossly lipemic samples. Avoid repeated freezing and thawing.

ASSAY PROCEDURE

Materials Provided

The Scantibodies Calcitonin Kit (Part No. 3KG556) is supplied with the following:

Description	Number
Calcitonin Standards Part Nos. 3CA090, 3CB090, 3CC090, 3CD090, 3CE090, 3CF090	6 vials
Calcitonin Control Serum Part Nos. 3CA091, 3CB091	2 vials
Anti-Calcitonin Coated Tubes Part No. 3KT005	2 packages of 50 tubes (100 tubes total)
^{125}I Anti-Calcitonin Part No. 3KL008	2 vials
Wash Concentrate Part No. 3KW001	1 vial
Directional Insert Part No. 3KI082	1 insert

Materials And Equipment Required But Not Provided:

Polystyrene or polypropylene tubes, 12 x 75 mm
Precision pipettors: 200 µL
Repeating dispenser: 100 µL
Test tube rack
Parafilm or equivalent for covering tubes
Distilled or deionized water
Vortex mixer
Aspiration device or suitable tube washer
Timer capable of timing 24 hours
Rotator, capable of maintaining 180 ± 20 RPM
Gamma counter calibrated to detect ^{125}I

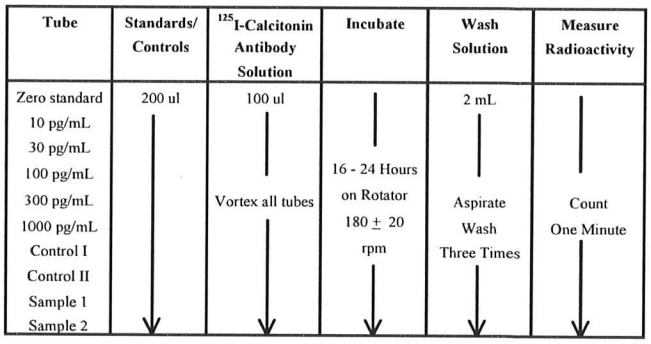
Preparation for Assay

For each assay, prepare the following groups of tubes and place them in a test tube rack (double determination): [Note: single determination is acceptable with sufficient experience]
2 total count tubes (use uncoated tubes)
2 tubes for each standard concentration
2 tubes for each control
2 tubes for each patient sample

Pipetting and Incubation Steps

1. Pipette 200 µL of standards, samples and control sera into the corresponding tubes. Pipette directly to the bottom of the tubes.
2. Pipette 100 µL of ^{125}I Anti-Calcitonin into each tube.
3. Mix tubes gently on the vortex mixer.
4. Cover the test tube rack with Parafilm or equivalent.
5. Incubate the tubes at room temperature (15 - 30° C) for 16-24 hours on a rotator at 180 ± 20 RPM.
6. Aspirate the contents of the tubes (except the total count tubes) and add 2 mL of the diluted wash solution. Aspirate the wash solution. Repeat this washing process until 3 washings are completed.
7. Count the washed tubes for one minute in a gamma counter.

Calcitonin Immunoassay Flow Chart



If data reduction requires total count tubes, label duplicate tubes (or a single tube) appropriately, pipet 100 uL of ^{125}I - Calcitonin Antibody Solution into each tube and cap.

PROCEDURAL COMMENTS

Known Interferences:

Grossly hemolyzed or lipemic samples.
Samples from patients receiving radioisotopes for diagnostic or therapeutic purposes.
Contamination of the sample or assay tube with ^{125}I or other radioisotopes.
Samples containing up to 250 mg/dL Triglycerides, 15 mg/dL Hemoglobin, and 15 mg/dL Bilirubin do not exhibit any effect on the assay within the medical decision point.

Reagents from different lot numbers must not be interchanged.

All reagents that are to be pipetted into tubes should be pipetted directly into the bottom of the tubes. This is to avoid losing liquid on the surface of the tube as the liquid runs down the tube.

The washing step is an important step in the assay procedure. Accurate dispensing of the wash solution and complete aspiration of the tube contents is essential to achieving assay sensitivity, low background and assay precision.

It is recommended that standards and patient samples be assayed in duplicate. The average counts per minute of each duplicate should then be used for data reduction and the calculation of results.

Avoid sample carryover contamination by using a new pipette tip for each sample.

CALCULATION OF RESULTS

Evaluation

The standard curve is generated using prepared Calcitonin standards. Refer to individual vial labels for exact concentrations. Generate the curve as follows:*

1. Calculate the average CPM for each pair of assay tubes.
2. The zero standard tubes are blanks that measure the non-specific binding (NSB) that may occur in this assay. Subtract the average CPM for each CPM of the zero standard tubes from the average of each calibrator and sample measurement. This is your corrected CPM.
3. The standard curve is prepared by plotting the corrected CPM of each standard level on the ordinate against the standard concentration on the abscissa using log-log paper.

*Computer assisted data reduction programs for IRMA assays may also be used for calculations of the Calcitonin Immunoassay. The four parameter logistics program, Cubic Spline is one of these methods[9].

Standard Calculation Example:

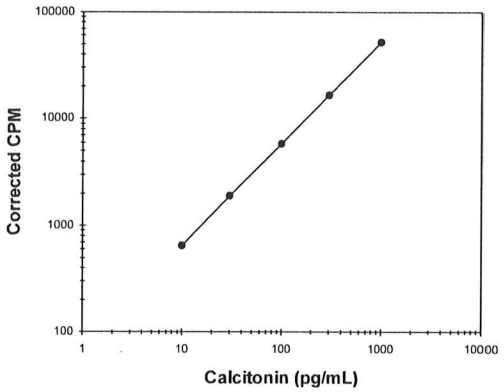
Average CPM of Zero Standard = 442
Average CPM of 10 pg/mL Standard = 1087
CPM (10 pg/mL) - CPM (0 pg/mL) = 1087 - 442
Corrected CPM = 645

Sample Data

Tube	CPM	Avg. CPM	Corrected CPM	Calcitonin (pg/ml)
Total Counts	261362 251421	256391		
zero std.	444 440	442		
10 pg/ml	1095 1080	1087	645	
30 pg/ml	2422 2283	2352	1910	
100 pg/ml	6268 6323	6295	5853	
300 pg/ml	17245 17102	17173	16731	
1000 pg/ml	53197 53711	53454	53012	
Control I	1676 1673	1674	1232	19
Control II	12187 12056	12121	11679	207

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

Representative Standard Curve



Automated data reduction can also be used to construct the Scantibodies Calcitonin Standard Curve. To program automated data reduction systems or to adapt an existing program consult the data processor manufacturer or the programmer.

QUALITY CONTROL

Control sera should be run with each batch of standards and patient samples. Results generated from the analysis of the control sera should be evaluated for acceptability using appropriate statistical methods. In assays where one or more of the control sera values lie outside the acceptable limits, the results for the patient sample may not be valid.

LIMITATIONS OF THE PROCEDURE

Like any analyte used as a diagnostic adjunct, Calcitonin results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

Immunoassays are optimized and calibrated for the determination of antigens in their intact and unaltered state. Genetic variations or degradation of antigens into subunits or other fragments may alter antibody binding characteristics and affect final results. Such samples may exhibit discordant results between different assays as the effect of such altered states is particular to each defined antibody assay.

No drugs have been investigated for assay interference.

EXPECTED VALUES

Scantibodies Laboratory recommends that each laboratory establish its own range of expected values. The Calcitonin level normal ranges were determined following the NCCLS guidelines (C28-A) in serum from 125 apparently healthy adults using the Scantibodies Calcitonin Immunoassay. The study consisted of 72 female and 53 male individuals. Consistent with the literature, Calcitonin levels were found to be generally lower in normal females than in normal males. The normal range for the 53 males was 3 to 21 pg/mL, whereas the normal value for 72 females was 1 to 8 pg/mL.

PERFORMANCE CHARACTERISTICS

Precision and Reproducibility

The precision (intra-assay variation) of the Scantibodies Calcitonin Immunoradiometric Assay was calculated from 20 replicate determinations on three serum samples in the same assay. The reproducibility (inter assay variation) of Calcitonin was determined by performing 20 different assays on three serum samples.

Intra-Assay Variation

Kit Batch	Sample	Mean Value (pg/mL)	Std. Dev. (pg/mL)	% Coefficient of Variation
E1	1	19.58	0.68	3.48
E1	2	189.37	3.33	1.76
E1	3	504.61	10.44	2.07
E2	1	16.93	0.37	2.17
E2	2	186.97	3.35	1.79
E2	3	511.59	9.10	1.78
E3	1	15.72	1.45	9.24
E3	2	178.52	8.93	5.00
E3	3	475.11	12.20	2.57

Note: Studies have demonstrated that there is less than a 10% change in precision at the end of the shelf life of the kit.

Inter-Assay Variation

Kit Batch	Sample	Mean Value (pg/mL)	Std. Dev. (pg/mL)	% Coefficient of Variation
E1	1	19.73	1.81	9.18
E1	2	188.09	14.05	7.47
E1	3	487.73	32.19	6.6
E2	1	16.48	0.38	2.33
E2	2	178.78	6.44	3.60
E2	3	466.79	18.27	3.91
E3	1	17.31	1.68	9.72
E3	2	180.97	7.69	4.25
E3	3	483.93	18.71	3.87

Sensitivity/Detection Limit

The detection limit of the assay is defined as the lowest measurable value distinguishable from zero. This sensitivity was determined by assaying the zero calibrator 20 times in the same assay. The detection limit determined is approximately 1 pg/mL for Calcitonin IRMA at 2 standard deviations above the mean of the CT zero standard.

The functional sensitivity is defined as being the measured concentration by imprecision profile for a CV equal to 20%. It has been assessed as being 1 pg/mL.

The highest concentration of calcitonin measurable without sample dilution is the concentration of the highest calibrator (~1,000 pg/mL).

High Dose Hook Effect

No high dose hook effect was observed up to 1000 pg/mL which is within the linear range of the assay. This was observed by testing the counts of spiked synthetic Calcitonin (1-32) from 100,000 pg/mL diluted sequentially to less than the lowest standard. At 12,500 pg/mL, the counts still yield a greater result than the highest standard.
It is recommended that all samples reading greater than 1,000 pg/mL be diluted 1:10 with the zero standard and repeated 10-fold as necessary until the result is repeatable.

Cross Reactivity Studies

Crossreactant	Concentration of Crossreactant	Calcitonin without Crossreactant (pg/mL)	Calcitonin with Crossreactant (pg/mL)	Change In Calcitonin (pg/mL)	Crossreactivity (%)
PTH	100,000 pg/mL 25,000 pg/mL 10,000 pg/mL	359.38 359.38 359.38	363.35 349.80 350.79	3.97 -9.58 -8.59	0.00397 -0.03832 -0.0859
TSH	5,000 µIU/mL 500 µIU/mL 50 µIU/mL	359.38 359.38 359.38	354.26 360.00 359.52	-5.12 0.62 0.14	-0.1024 0.124 0.28
Calcitonin Gene Related Peptide (CGRP)	1,000,000 pg/mL 100,000 pg/mL	359.38 359.38	353.28 363.08	-6.1 3.7	0.0061 0.0037
Porcine Calcitonin	1,000,000 pg/mL 100,000 pg/mL	359.38 359.38	350.17 365.33	-9.21 5.95	-0.000921 0.00595
Salmon Calcitonin	1,000,000 pg/mL 100,000 pg/mL	359.38 359.38	311.62 349.80	-47.76 -9.58	-0.004776 -0.00958

Summary:

Each crossreactant is spiked into a sample containing Calcitonin. Calcitonin level is measured before and after the spike.

Conclusion:

There is no correlation between the concentration increase of the spiked crossreactant and the concomitant change in measured Calcitonin concentration. Hence, it can be concluded that none of the crossreactants interfere with this Calcitonin IRMA in the concentrations of crossreactants tested.

Analytical Recovery

Various amounts of Calcitonin were added to four different patient sera to determine the recovery. The results are described in the following table:

Sample ID	Endogenous Calcitonin (pg/mL)	Added Calcitonin Theoretical (pg/mL)	Measured Calcitonin Theoretical (pg/mL)	Assayed Calcitonin Value (pg/mL)	Recovery (%)
Sample 1	33.16		33.16	33.16	–
	33.16	+30.77	63.93	68.83	108
	33.16	+100.27	133.43	134.79	101
Sample 2	170.35		170.35	170.35	–
	170.35	+32.19	202.54	207.18	102
	170.35	+108.37	278.72	267.00	96
Sample 3	410.26		410.26	410.26	–
	410.26	+63.40	473.66	494.35	104
	410.26	+210.83	621.09	678.47	109
Sample 4	3.56		3.56	3.56	–
	3.56	+950.00	953.56	988.54	104

Linearity of Patient Sample Dilutions: Parallelism

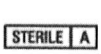
Six patient serum samples were diluted with the zero standard. Results in pg/mL are shown below:

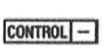
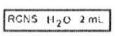
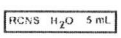
Sample ID	Dilution	Assayed Calcitonin Value (pg/mL)	Results Corrected for Dilution Factor (pg/mL)	Recovery (%)
Sample 1	undiluted	54.81	54.81	--
	1:2	28.24	56.48	103
	1:4	14.82	59.28	108
Sample 2	undiluted	261.76	261.76	--
	1:2	153.81	307.62	118
	1:4	79.50	318.00	121
	1:8	35.44	283.52	108
	1:16	17.04	272.64	104
Sample 3	undiluted	949.99	949.99	--
	1:2	589.96	1179.92	124
	1:4	322.23	1288.92	136
	1:8	165.95	1327.60	140
	1:16	81.44	1303.04	137
	1:32	39.1	1251.20	132
	1:64	18.09	1157.76	122
	1:128	9.43	1207.04	127
Sample 4	undiluted	40.99	40.99	--
	1:2	18.02	36.04	88
	1:4	10.28	41.12	110
Sample 5	undiluted	181.30	181.30	--
	1:2	99.75	199.50	110
	1:4	41.51	166.04	92
	1:8	18.66	149.28	82
	1:16	10.21	163.36	90
Sample 6	undiluted	523.46	523.46	--
	1:2	336.56	673.12	129
	1:4	182.14	728.56	139
	1:8	89.34	714.72	137
	1:16	36.73	587.68	112
	1:32	16.28	520.96	100

Correlation:

Two hundred forty-eight (248) normal serum samples, some of which were spiked with calcitonin, were analyzed for calcitonin using both the Scantibodies immunoradiometric assay and a commercially available calcitonin immunoradiometric assay. Calcitonin determinations in both assays were performed on the same day. A correlation coefficient (R) of 0.98 was obtained with a slope of 1.06 and an intercept of -1.48 where x represents the predicate device data and y represents the SLI calcitonin tube assay. Calculations were made with serum samples ranging from 1.00 pg/mL– 951 pg/mL.

Component Chemical Characterization:	1) Antibodies coated on to polystyrene tubes.
	2) Radioactive Isotope containing Iodine-125 with radioactivity <10 μCi and Sodium Azide @ 0.1%.
	3) Calibrators & Controls – Human Serum containing Sodium Azide @ 0.1%
	4) Wash Concentrate containing sodium azide @ 1.5%.
Hazardous Ingredients:	Radioactive Isotope (Iodine-125) @ <10 μCi/Vial (<370 kBq) CAS Number: 7553-56-2 Symbols: Harmful Xn R-phrases: R22, R52/53 S-phrases: S28, S45, S53, S60, S61
	Sodium Azide @ 0.1% CAS Number: 026628-22-8 Symbols: N/A R-phrases: N/A S-phrases: N/A
	Sodium Azide @ 1.5% CAS Number: 026628-22-8 Symbols: Very Toxic T+; N R-phrases: R28, R32, R50/53 S-phrases: S28, S45, S53, S60, S61

Symbol	Used for	Symbol	Used for
	Do Not Reuse		Use By YYYY-MM-DD or YYYY-MM
	Batch Code		Serial Number
	Date of Manufacture		Sterile
	Sterilized Using Aseptic Processing Technique		Catalog Number
	Caution, Consult Accompanying Documents		Biological Risks

Symbol	Used for	Symbol	Used for
	Manufacturer		Authorized Representative in the European Community
	Contains Sufficient for < n > Tests		For IVD Performance Evaluation Only
	*In vitro* Diagnostic Medical Device		Upper Limit of Temperature
	Lower Limit of Temperature		Temperature Limitation
	Consult Instructions for Use		Positive Control
	Control		Negative Control
	Antibody Coated Beads		Antibody Coated Tubes
	Radioactive Iodine Tracer		Wash Solution
	Reconstituted with 2 mL Water		Reconstituted with 5 mL Water
	European Conformity Mark		Radioactive
	Toxic		Harmful
	Corrosive		Oxidizing

CALCITONIN BIBLIOGRAPHY

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