Snibe Diagnostic

CE 0123 REF 130261001M:100 tests/kit
130661001M: 50 tests/kit
130761001M: 30 tests/kit

# MAGLUMI® Intact PTH (CLIA)

## INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Parathyroid Hormone (PTH) in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in assessment of parathyroid function and in the differential diagnosis of hypercalcemia and hypocalcemia.

## SUMMARY

Parathyroid Hormone (PTH) is an 84-amino acid single-chain polypeptide that is synthesized and secreted by chief cells of the parathyroid glands[1]. The half-life of circulating intact PTH was less than 5 min[2]. The principal effect of PTH is to increase the extracellular fluid (ECF) calcium concentration by mobilizing calcium from bone, increasing tubular calcium reabsorption, and, indirectly on the gut, by increasing calcitriol synthesis. The principal effect of calcitriol is to increase intestinal absorption of calcium, but it also exerts negative regulatory control of PTH synthesis and further calcitriol synthesis[1]. PTH is a key calcium regulating hormone essential for calcium homeostasis, vitamin D-dependant calcium absorption, renal calcium reabsorption and renal phosphate clearance[3]. In the kidney, PTH stimulates renal reabsorption of calcium and promotes phosphate excretion[4].

Hypoparathyroidism is due to deficient or absent parathyroid hormone[5]. When the actions of PTH are reduced or lost, all subsequent steps in the maintenance of homeostasis are impaired, resulting in hypocalcemia, hyperphosphatemia, and hypercalciuria[4]. Hypocalcemia has a defined role in the pathogenesis of renal osteodystrophy[6]. Hypercalcemia results in decreased PTH secretion, increased intracellular degradation of PTH in chief cells, and decreased PTH synthesis. Serum ionized calcium concentration is increased in primary hyperparathyroidism but is usually normal or low in patients with chronic renal failure[1]. PTH causes phosphate loss through the kidneys. Thus, in patients with PTH-mediated hypercalcemia, serum phosphate levels tend to be low. Chronic renal failure generally causes hypocalcemia. If untreated, prolonged high phosphate and low vitamin D levels can lead to increased PTH secretion and subsequent hypercalcemia [7]. Secondary hyperparathyroidism is a common complication of chronic kidney disease, characterized by parathyroid hyperplasia and persistently elevated levels of parathyroid hormone[8]. It is likely that increased PTH secretion in patients with renal secondary hyperparathyroidism is primarily caused by parathyroid gland hyperplasia[1]. Daily subcutaneous injections of relatively low doses of parathyroid hormone stimulate bone formation in osteopenic animals and osteoporotic women and men[9].

## TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, ABEI labeled with PTH monoclonal antibody, buffer and magnetic microbeads coated with PTH monoclonal antibody are mixed thoroughly and incubated, reacting to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of PTH present in the sample.

## REAGENTS

**Kit Contents**

| Component | Description | 100 tests/kit | 50 tests/kit | 30 tests/kit |
|---|---|---|---|---|
| **Magnetic Microbeads** | Magnetic microbeads coated with PTH monoclonal antibody (~6.67 μg/mL) in PBS buffer, $NaN_3$ (<0.1%). | 2.5 mL | 1.5 mL | 1.0 mL |
| **Calibrator Low** | A low concentration of PTH antigen in PBS buffer, $NaN_3$ (<0.1%). | 1.5 mL | 1.5 mL | 1.5 mL |
| **Calibrator High** | A high concentration of PTH antigen in PBS buffer, $NaN_3$ (<0.1%). | 1.5 mL | 1.5 mL | 1.5 mL |
| **Buffer** | Tris-HCl buffer, $NaN_3$ (<0.1%). | 6.5 mL | 4.0 mL | 3.0 mL |
| **ABEI Label** | ABEI labeled with PTH monoclonal antibody (~500 ng/mL) in Tris-HCl buffer, $NaN_3$ (<0.1%). | 7.5 mL | 4.5 mL | 3.3 mL |
| **Control 1** | A low concentration of PTH antigen (40.0 pg/mL) in PBS buffer, $NaN_3$ (<0.1%). | 1.5 mL | 1.5 mL | 1.5 mL |
| **Control 2** | A high concentration of PTH antigen (400 pg/mL) in PBS buffer, $NaN_3$ (<0.1%). | 1.5 mL | 1.5 mL | 1.5 mL |
| All reagents are provided ready-to-use. | | | | |

**Warnings and Precautions**

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

**Reagent Handling**

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

**Storage and Stability**

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

| Stability of the Reagents | |
|---|---|
| Unopened at 2-8°C | until the stated expiration date |
| Opened at 2-8°C | 6 weeks |
| On-board | 4 weeks |

| Stability of Controls | |
|---|---|
| Unopened at 2-8°C | until the stated expiration date |
| Opened at 10-30°C | 6 hours |
| Opened at 2-8°C | 6 weeks |
| Frozen at -20°C | 3 months |
| Frozen and thawed cycles | no more than 3 times |

## SPECIMEN COLLECTION AND PREPARATION

**Specimen Types**

Only the specimens listed below were tested and found acceptable.

| Specimen Types | Collection Tubes |
|---|---|
| **Serum** | Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel. |
| **Plasma** | K2-EDTA, Na-heparin |

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

**Specimen Conditions**

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.
- Because of the short half-life of PTH, it is recommended that, the blood should be centrifuged immediately.

**Preparation for Analysis**

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 100 μL.

**Specimen Storage**

Specimens removed from the separator, red blood cells or clot may be stored up to 8 hours at 10-30°C or 2 days at 2-8°C, or 6 months frozen at -20°C. Frozen specimens subjected to up to one freeze/thaw cycle have been evaluated.

**Specimen Shipping**

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

**Specimen Dilution**

- Samples, with Intact PTH concentrations above the analytical measuring interval, can be diluted with manual dilution procedure. The recommended dilution ratio is 1:10. The concentration of the diluted sample must be >500 pg/mL.
- For manual dilution, multiply the result by the dilution factor.
- Please choose applicable diluents or ask Snibe for advice before manual dilution.

## PROCEDURE

**Materials Provided**

Intact PTH (CLIA) assay, control barcode labels.

**Materials Required (But Not Provided)**

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000, Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

**Assay Procedure**

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

**Calibration**

Traceability: This method has been standardized against the WHO International Standard 95/646.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 28 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

**Quality Control**

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[10].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the Intact PTH assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi systems and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Intact PTH (CLIA) Controls (REF: 160201474MT) from Snibe or our authorized distributors for more.

## RESULTS

**Calculation**

The analyzer automatically calculates the Intact PTH concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in pg/mL. For further information please refer to the Analyzer Operating Instructions.

**Interpretation of Results**

The expected range for the Intact PTH assay was obtained by testing 630 apparently healthy individuals in China, gave the following expected value:
15-65 pg/mL ($2.5^{th}$-$97.5^{th}$ percentiles).
Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

## LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the Intact PTH results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- The assay is mainly used to aid in assessment of individuals with suspected or confirmed parathyroid dysfunction and in the differential diagnosis of hypercalcemia and hypocalcemia in individuals patients.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[11,12]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[13].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

## SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

**Precision**

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

| Sample | Mean (pg/mL) (n=180) | Within-Run | | Between-Run | | Reproducibility | |
|---|---|---|---|---|---|---|---|
| | | SD (pg/mL) | %CV | SD (pg/mL) | %CV | SD (pg/mL) | %CV |
| Serum Pool 1 | 14.965 | 0.472 | 3.04 | 0.229 | 1.48 | 0.785 | 5.06 |
| Serum Pool 2 | 63.840 | 1.640 | 2.57 | 0.730 | 1.14 | 2.557 | 4.01 |
| Serum Pool 3 | 397.952 | 5.555 | 1.40 | 4.382 | 1.10 | 10.087 | 2.53 |
| Plasma Pool 1 | 15.139 | 0.447 | 2.95 | 0.249 | 1.64 | 0.691 | 4.56 |
| Plasma Pool 2 | 64.841 | 1.636 | 2.52 | 0.200 | 0.31 | 2.563 | 3.95 |
| Plasma Pool 3 | 399.359 | 5.159 | 1.29 | 4.084 | 1.02 | 13.607 | 3.41 |
| Control 1 | 40.284 | 0.976 | 2.42 | 0.620 | 1.54 | 1.661 | 4.12 |
| Control 2 | 398.453 | 5.730 | 1.44 | 3.004 | 0.75 | 9.539 | 2.39 |

**Linear Range**

5.00-5000 pg/mL (defined by the Limit of Quantitation and the maximum of the master curve).

**Reportable Interval**

3.00-50000 pg/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

**Analytical Sensitivity**

Limit of Blank (LoB) =1.00 pg/mL.
Limit of Detection (LoD) =3.00 pg/mL.
Limit of Quantitation (LoQ) =5.00 pg/mL.

**Analytical Specificity**

**Interference**

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

| Interference | No interference up to | Interference | No interference up to |
|---|---|---|---|
| Hemoglobin | 500 mg/dL | K2-EDTA | 22.75 μmol/mL |
| Intralipid | 5000 mg/dL | Heparin sodium salt | 80 IU/mL |
| Bilirubin | 65 mg/dL | Acetaminophen | 20 mg/dL |
| HAMA | 40 ng/mL | Ibuprofen | 500 μg/mL |
| ANA | 398 AU/mL | Disodium pamidronate | 10 μg/mL |
| Rheumatoid factor | 2000 IU/mL | 2,6-Diisopropylphenol | 2 μg/mL |
| Biotin | 0.5 mg/dL | Acetylsalicylic Acid | 650 μg/mL |
| Total protein | 10 g/dL | Salicylic acid | 600 μg/mL |

**Cross-Reactivity**

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactant in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:



| Cross-reactant | No interference up to | Cross-reactant | No interference up to |
|---|---|---|---|
| C-Telopeptide | 200 ng/mL | PTH 1-44 | 100 ng/mL |
| Human calcitonin | 200 ng/mL | PTH 39-68 | 200 ng/mL |
| Osteocalcin | 200 ng/mL | PTH 53-84 | 200 ng/mL |
| Bone Specific Alkaline Phosphatase | 3000 pg/mL | PTH 44-68 | 200 ng/mL |
| PTH 1-34 | 200 ng/mL | PTH 39-84 | 200 ng/mL |

**High-Dose Hook**

No high-dose hook effect was seen for Intact PTH concentrations up to 150000 pg/mL.

**Method Comparison**

A comparison of the Intact PTH assay with a commercially available immunoassay, gave the following correlations (pg/mL):
Number of samples measured: 118
Passing-Bablok: $\hat{y}$=1.0048 $x$+0.5517, τ=0.976.
The clinical specimen concentrations were between 8.93 and 4971 pg/mL.

## REFERENCES


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## SYMBOLS EXPLANATIONS

| | | | |
|---|---|---|---|
|  | Consult instructions for use |  | Manufacturer |
|  | Temperature limit (Store at 2-8°C) |  | Use-by date |
|  | Contains sufficient for<n> tests |  | Keep away from sunlight |
|  | This way up | EC REP | Authorized representative in the European Community |
| IVD | *In vitro* diagnostic medical device | CONTENTS | Kit component |
| REF | Catalogue number | LOT | Batch code |
| CE 0123 | CE marking with notified body ID number | | |



**Shenzhen New Industries Biomedical Engineering Co., Ltd.**
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax:+86-755-28292740

EC REP
**Shanghai International Holding Corp. GmbH (Europe)**
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726