Active® PTHrP IRMA

REF DSL8100

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Immunoradiometric kit for the quantitative measurement of parathyroid hormone-related peptide (PTHrP) in human plasma. For research use only - not for use in diagnostic procedures

PRINCIPLE

The 2-site immunoradiometric (IRMA) assay for parathyroid hormone-related peptide (PTHrP) is a sandwich-type assay. In the kit, polyclonal antibodies directed against two different epitopes of PTHrP are used. Samples or calibrators are incubated in tubes pre-coated with one polyclonal antibody, and with a second liquid 125I-labeled polyclonal antibody. After incubation, unbound reagents are removed by washing the tubes. The amount of 125I-labeled anti-PTHrP bound to the tube is directly proportional to the concentration of PTHrP present in the sample. A standard curve is constructed and unknown PTHrP values are obtained from the curve by interpolation.

For Summary and Explanation of the Test see APPENDIX.

WARNING AND PRECAUTIONS

General remarks:

- The vials with calibrators and controls should be opened as shortly as possible to avoid evaporation.
- Do not mix the reagents from kits of different lots.
- Do not use any component beyond the expiration date shown on its label.
- A standard curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Calibrators and controls should be mixed before use by inverting or swirling gently rather than vortexing.

Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material are subject to the regulations of the country of use. Adherence to the basic rules of radiation safety should provide adequate protection:

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipeting by mouth.
- Avoid all contact with radioactive materials by using gloves and lab coat.
- All manipulation of radioactive substances should be done in an appropriate location, away from corridors and other busy areas.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.

Sodium azide

Some reagents contain sodium azide as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up [1].

Materials of human origin

Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

The Materials Safety Data Sheet (MSDS) is available upon request.

GHS HAZARD CLASSIFICATION

Calibrators / Controls — DANGER

H360	May damage fertility or the unborn child.
P201	Obtain special instructions before use.
P280	Wear protective gloves, protective clothing and eye/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
	Boric Acid 0.1 - 0.3%
	Sodium Borate Decahydrate 0.1 - 0.5%

SDS Safety Data Sheet is available at techdocs.beckmancoulter.com

SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

- EDTA plasma is the recommended sample type.
- Blood samples should be collected in the presence of protease inhibitors. The following combination of protease inhibitors has been shown to be effective [2]:

aprotinin	500 kU/L
leupeptin	2.5 mg/L

- Blood samples should be separated by centrifugation within two hours of collection and the plasma should be immediately assayed or frozen and stored at -70°C or lower. It is recommended to prepare aliquots to avoid repeated freezing and thawing.
- Frozen samples should be thawed and mixed thoroughly by gentle swirling or inversion prior to use.
- Plasma samples stored at -70 °C should be assayed within 30 days of the collection date [3].

MATERIALS PROVIDED

All unopened reagents in the kit are stable until the expiration date indicated on the kit label, when stored at 2-8°C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

Storage conditions for opened reagents are indicated in appropriate paragraphs.

Anti-PTHrP antibody coated tubes: 2 x 50 tubes (ready-to-use)

Plastic tubes with anti-human PTHrP antibody immobilized to the inside wall of each tube.

The tubes are packaged into protective bag with silica-gel. It is strongly recommended to store tubes all time before the usage in this tightly bag.

Note: The process which is used to coat the tubes with antibody sometimes causes a crystalline substance to form on the inside walls of the tubes. This will not affect the performance of the assay in any way.

^{125}I –labeled anti-PTHrP tracer (YELLOW): one 11 mL vial (ready-to-use)

At the time of manufacture, the vial contains 370 kBq, (<10 µCi) of 125I-labeled anti-PTHrP antibody in buffer with proteins, sodium azide (<0.1%) and a dye.

Calibrators: five vials labeled 0-4 (lyophilized)

The calibrator vials contain from 0 to approximately 2,000 pg/mL (0 to approximately 202 pmol/L) of PTHrP (1-86) in buffer with proteins and sodium azide (<0.1%). The exact concentration is indicated on each vial

label. The volume for reconstitution is indicated on the vial label. **Use immediately. Discard after use.**

The calibrator values were established using an internal standard.

Controls: two vials labeled 1, 2 (lyophilized)

The vials contain PTHrP in buffer with proteins and sodium azide (<0.1%). The expected values are indicated in a supplement found in the kit. The volume for reconstitution is indicated on the vial label. **Use immediately. Discard after use.**

MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- 12 x 75 mm plastic uncoated test tubes for Total counts.
- Test tube rack for 12 x 75 mm tubes.
- Deionized water.
- Precision micropipets (100 µL, 200 µL).
- Semi-automatic pipets (100 µL, 3 mL).
- Vortex type mixer.
- Shaker capable of ≥ 180 rpm.
- A sponge rack or similar device for decantation.
- Absorbent material for blotting tubes.
- Gamma counter set for 125 iodine.
- Aspiration system.
- Log-log graph paper or computer with IRMA data analysis program.

PROCEDURE

Preparation of reagents

Let all the reagents come to room temperature and mix them thoroughly by gentle inversion before use.

Reconstitution of calibrators and control sera

The content of the vials is reconstituted with the volume of distilled water indicated on the vial label. Mix gently to avoid foaming before dispensing. After reconstitution, use immediately. Discard after use.

Assay procedure

Step 1 Additions*	Step 2 Incubation	Step 3 Washing & Counting
To antibody coated tubes successively add:	Incubate 16 - 22 hours at room temperature (18 - 25°C) on a shaker set at ≥180 rpm.	Aspirate or decant all tubes, (except «total cpm» tubes), by simultaneous inversion with a sponge rack into a radioactive waste receptacle.
200 µL of calibrator, control or sample		After decanting strike the tubes sharply on absorbent material to facilitate complete drainage and then allow them to drain on the absorbent material for 1-2 minutes. Blot the tubes.
and immediately add 100 µL of tracer.		Wash three times with 3 mL of deionized water (except «total cpm» tubes). Aspirate or decant and blot after each wash step.
Vortex gently for 1-2 seconds.		Count bound cpm (B) and total cpm (T) for 1 minute.

*Add 100 µL of tracer to 2 additional tubes to obtain total cpm.

RESULTS

Results are obtained from the standard curve by interpolation. The curve is used for the determination of PTHrP concentrations in samples measured at the same time as the calibrators.

Standard curve

The results in the quality control department were calculated using *spline* curve fit with determined radioactivity *(cpm_{cal}-cpm_{cal0})* on the log vertical axis and analyte concentration of the calibrators on the log horizontal axis (pg/mL).

Other data reduction methods may give slightly different results.

Total activity: 138,501 cpm				
Calibrators	**PTHrP (pg/mL)**	**cpm (n=3)**	**B/T (%)**	**cpm_{cal} - cpm_{cal0}**
0	0	188	-	-
1	32	844	0.47	656
2	225	4,088	2.82	3,900
3	590	7,439	5.24	7,251
4	2,700	18,970	13.56	18,782

(Example of standard curve, do not use for calculation).

Samples

For each sample, locate the cpm or B/T value on the vertical axis and read off the corresponding analyte concentration on the horizontal axis.

To convert concentrations from pg/mL to pmol/L, multiply results by 0.101

EXPECTED VALUES

Each laboratory should establish its own reference ranges.

QUALITY CONTROL

Good laboratory practices require that control samples be used regularly to ensure the quality of the results obtained. These controls must be processed in exactly the same way as the patient samples, and it is recommended that their results be analyzed using appropriate statistical methods.

Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling, or deterioration of reagents. In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: imunochem@beckman.com

In the US, contact the Beckman Coulter technical support at 1-800-854-3633; or by email at: immunoassay@beckman.com

PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Sensitivity

Analytical sensitivity: 7.43 pg/mL

Functional sensitivity: 13.93 pg/mL

Specificity

The antibodies used in the immunoassay are highly specific for PTHrP.

Precision

Intra-assay

Samples were assayed 25 times in the same run. The coefficients of variation were ≤ 7.6%.

Inter-assay

Samples were assayed in duplicate in 10 different runs. The coefficients of variation were ≤ 17.0%.

Accuracy

Dilution test

The dilution of patient's samples is not recommended. Results may be affected by poor stability of PTHrP. Moreover, dilution can be non-linear in some samples.

Measurement range (from analytical sensitivity to highest calibrator):

7.43 to approximately 2,000 pg/mL.

LIMITATIONS

- Failure to follow these instructions for use (IFU) may significantly affect results.
- Failure to blot tubes adequately following decantation may result in poor replication and spurious values.
- Hemolyzed, icteric and lipemic specimens may give false values and should be avoided.

- The possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

Hook effect

No hook effect was observed until 5,000 pg/mL.

APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Summary and explanation of the test

Parathyroid hormone (PTH) acts directly on bone to promote resorption and upon the kidney to decrease calcium excretion, thereby raising calcium levels. It is also known to cause increased renal production of cyclic adenosine monophosphate (cAMP), which often occurs in patients with low or undetectable plasma PTH levels. Thus, when studies revealed that PTH sometimes could not be detected in either plasma or tumor extracts of a number of hypercalcemic patients whose tumor extracts resorbed bone in vitro, it was established that the tumors were producing something which was not PTH itself, but which had remarkably similar effects to PTH [4]. Also, because of evidence that PTH, itself, is often not responsible for hypercalcemia of malignancy (HM), peptides with PTH-like activity were implicated as possible mediators of HM [5]. The resulting search for such a substance led to the discovery of PTH-like biological activity in extracts of tumors of patients with HM, in culture medium from a tumor cell culture of such a patient, and in extracts of cancers from animal models of the syndrome [4]. Thus, parathyroid hormone-related peptide (PTHrP) was discovered.

PTHrP is produced by a variety of cancers and is thought to be responsible for the clinical syndrome of HM [5]. PTHrP and PTH are homologous in that eight of the first thirteen amino acid residues are identical, but, thereafter, PTHrP is unique [6]. This homology allows PTHrP to act via the parathyroid hormone receptor [7]. The PTH-like bioactivity of PTHrP, like that of PTH, is contained within the first 34 amino acids [8]. Based on chromosomal localization data and shared organizational features, the PTH and PTHrP genes appear to have arisen via an ancient duplication event and to represent members of a gene family. After this duplication event, the two genes have clearly evolved separately, and the PTHrP gene has developed an organization that is considerably more complex than is that of the PTH gene [9]. PTHrP appears to be involved in several different aspects of the reproductive process. There is evidence that PTHrP may help to regulate calcium metabolism during fetal development and adult life in man. In the fetus, PTHrP has been identified in epithelium from many sites and in parathyroid glands [7].

It has also been shown to be capable of stimulating the placental calcium pump that maintains the calcium gradient which is required for normal mineralization of the fetal skeleton [9]. Studies with a fetal lamb suggest that PTHrP is responsible for maintenance of this placental calcium gradient [4]. Tissues in which PTHrP has been identified include kidney, bone, smooth and skeletal muscle in the fetus, placenta, lactating breast, brain, pancreas, ovary, uterus, testis, and spleen [7].

Specificity

Data on cross-reactivity with several compounds are presented in the following table:

COMPOUND	% CROSS-REACTIVITY
C-Terminal PTH	ND
Mid-Molecule PTH	ND
Sex Hormone Binding Globulin	ND
Osteocalcin	ND
Calcitonin	ND
Insulin	ND
Erythropoietin	ND
PTH (39-84)	ND
Intact PTH	ND
Beta-2-microglobulin	ND

ND = Non-detectable (<0.1%)

Precision

Intra-assay

The intra-assay precision was determined from the mean of 25 replicates each.

EDTA Plasma	P1	P2	P3
Number of determinations	25	25	25
Mean (pg/mL)	89.55	465.9	1,732
C.V., (%)	4.55	7.56	7.34

Inter-assay

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

EDTA Plasma	P1	P2	P3
Number of determinations	10	10	10
Mean (pg/mL)	24.12	149.6	1,004
C.V., (%)	16.98	7.28	15.52

^{125}I Characteristics

$T_{1/2}$ (^{125}I) = 1443 h = 60.14 d

125I	E (MeV)	(%)
Y	0.035	
X	0.027	114
	0.032	25

Symbols Key

REF — Product Reference / Référence du produit / Produktreferenz / Riferimento prodotto / Número de referencia del producto / Referência do produto / Produktreferens / Κωδικός αναφοράς προϊόντος / 产品参考 / Gaminio nuoroda / Termékszám / Dane referencyjne produktu / Reference k produktu / Referenčné označenie výrobku / 제품 참조 자료 / Ürün Referansı / Ссылка на продукт / Референция за продукт / 產品參考

IVD — In Vitro Diagnostic / Diagnostic in vitro / In-vitro-Diagnostikum / Diagnostica in vitro / Para diagnóstico in vitro / Diagnóstico in vitro / InVitro-diagnostik / Για διάγνωση in vitro / 体外诊断 / In vitro diagnostika / In vitro diagnosztikai felhasználásra / Diagnostyka in vitro / Diagnostika in vitro / 체외 진단 / In Vitro Diagnostik / Диагностика in vitro / За ин витро диагностика / 體外診斷

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Radioactive / Radioactif / Radioaktiv / Radioattivo / Radiactivo / Radioactivo / Radioaktivt / Ραδιενεργό / 放射性 / Radioaktyvioji medžiaga / Radioaktív / Radioaktywny / Radioaktivní / Rádioaktívny / 방사성 / Radyoaktif / Радиоактивный / Радиоактивен / 具放射性

Ag 125I / Ab 125I — Tracer / Traceur / Tracer / Marcato / Trazador / Marcador / / Ανιχνευτής / 追踪剂 / Atsekamoji medžiaga / Nyomjelző / Znacznik / Radioindikátor / Indikátor (tracer) / 트레이서 / Tracer´lar / метка / Индикатор / 追蹤劑

CAL / CAL 0 — Calibrator / Calibrateur / Kalibrator / Calibratore / Calibrador / Calibrador / Kalibrator / Βαθμονομητής / 校准品 / Kalibravimo medžiaga / Kalibrátor / Kalibrator / kalibrátor / Kalibrátor / 보정물질 / Kalibratör / Калибратор / Калибратор / 校正液

CTRL — Control / Contrôle / Kontrolle / Controllo / Control / Controlo / Kontrolle / Μάρτυρας / 质控品 / Kontrolinė / Kontroll / Kontrola / Kontrola / Kontrola / 정도관리 / Kontrol / Контроль / Контролна / 質控品

TUBE — Tubes / tubes / Röhrchen / provette / tubos / Tubos de amostra / Provrör / σωληνάρια / 试管 / Mėgintuvėliai / Csövek / Probówki / Zkumavky / Skúmavky / 튜브 / Tüpler / пробирки / Епруветки / 試管

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28 May 2020

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