ARK-780CTACE210301

hPRL [I-125] IRMA KIT

(REF: RK-780CT)

The hPRL [^{125}I] IRMA system provides a direct quantitative *in vitro* determination of human prolactin in human serum. hPRL can be assayed in the range of 0-170 ng/mL using 100 µl serum samples.

Introduction

Human Prolactin (hPRL) is a protein hormone with a molecular weight of 22000 daltons. Prolactin is produced by the anterior pituitary and its secretion is under hypothalamic control. Prolactin release is inhibited by prolactin-inhibiting factor (PIF) and it has been shown to be stimulated by TRH.
The major physiologic action of Prolactin is the initiation and maintenance of lactation. Hyperprolactinemia is a common cause of infertility and gonadal dysfunction in women and men. Measurement of Prolactin is also of interest in the evaluation and management of patients with amenorrhea and galactorrhea. There are various physiological and pathological conditions affecting Prolactin levels: high concentrations are observed during pregnancy, lactation and stress. Prolactin is increased by some psychotropic agents and antihypertensive drugs and is decreased by the administration of L-dopa and bromocriptine.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.
The ^{125}I labelled signal-antibody binds to an epitope of the PRL molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".
During 1-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.
The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hPRL, the unknown concentration of hPRL in patient samples can determined

Contents of the kit

1. 1 bottle of TRACER (21 mL), ready to use, containing 740 kBq ^{125}I-anti-hPRL and capture anti-hPRL in buffer with red dye and 0.1 % NaN_3.
2. 6 vials of STANDARDS (1 mL) lyophilized, in bovine serum with 0.1 % NaN_3. The concentrations of standards are specified in the labels and in the quality certificate enclosed. See *Preparation of reagents.*
3. 1 vial of CONTROL SERUM (1 mL). Lyophilized human serum with 0.1 % NaN_3..
The concentration of the control serum is specified in the quality certificate enclosed. See *Preparation of reagents*.
4. 2 boxes of COATED TUBES, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
5. 1 bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN_3. See *Preparation of reagents.*
Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter
Recommended tools and equipment
repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-mL pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C) up to 4 months. Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens. Samples with a hPRL concentration higher than that of the most concentrated standard should be diluted and reassayed.

Preparation of reagents, storage

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. After dilution wash buffer can be stored at 2-8°C until expiry date.
Add 1000 µl distilled water to the lyophilized standards and control serum. Mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. After reconstitution standards and control can be stored below -20°C until expiry date.
Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!
Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S1-S6), control serum and samples. Optionally, label two test tubes for total counts (T).
2. Pipette 100 µl of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
3. Pipette 200 µl of tracer into each tube.
4. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
5. Incubate tubes for 1 hour, shaking at room temperature.
6. Add 2.0 mL of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside-down position place the rack on an absorbent paper for 2 minutes.
7. Return the tube-rack to an upright position, and repeat step-6 one more time.
8. Count each tube for at least 60 seconds in a gamma counter.
9. Calculate the PRL concentrations of the samples as described in calculation of results or use special software.

Table I. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**100**		
Control			**100**	
Sample				**100**
Tracer	**200**	**200**	**200**	**200**
Shake for 1 hour at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.
Calculate the average count per minute (CPM) for each pair of assay tubes.
Calculate the normalized percent binding for each standard (S), control (C) and sample (M) respectively by using the following equation:

$$B/T(\%) = \frac{S_{2\text{-}6} / C / M_x \text{ (cpm)} - S_1 \text{ (cpm)}}{T(\text{cpm})} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of PRL.
Determine the PRL concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Table II. Typical assay data

Tubes	hPRL (ng/mL)	Mean cpm	B/T%
T		278224	
S1	0	59	0.02
S2	2	2277	0.82
S3	7	7169	2.58
S4	18	19278	6.96
S5	52	54597	19.7
S6	160	137653	49.7
C	7.3	7515	2.7

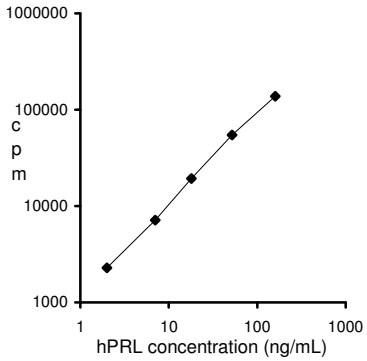

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples*)

Characterization of assay

Calibration

1 ng of the calibrator preparation is equivalent to 35 µIU NIBSC 97/714 reference reagent.

Sensitivity

A detection limit of 0.04 ng/mL has been obtained by assaying 20 duplicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Specificity

The monoclonal antibodies used in this IRMA kit are specific for hPRL. No cross reactivity with hPL, hGH can be detected in normal physiological concentrations.

Precision

Prolactin serum pools at 3 levels were assayed in 20 replicates to determine intra-assay precision. Values obtained are shown below.

Pool	Mean ng/mL	SD	CV %
1	6.36	0.31	4.82
2	12.21	0.57	4.67
3	27.69	0.79	2.86

Reproducibility

To determine inter-assay reproducibility 3 serum pools were measured in duplicates in 18 independent assays. Values obtained are shown below.

Pool	Mean ng/mL	SD	CV %
1	6.89	0.53	7.74
2	15.77	1.54	9.74
3	31.57	2.33	7.37

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking 5 serum samples with known amount of hPRL at 3 levels. The recovery results obtained were between 97% and 107% (mean = 102.6%).

Dilution test (linearity)

Five samples were measured in a series of dilution with zero-standard. The recovery after dilution was between 98% and 108%. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$Y = 1.0242X - 0.0724 \quad R^2 = 0.9989 \quad n = 20$

Expected Values

It is recommended that each laboratory determine a reference range for its own patient population.

	Men	Women	Menopause
Samples	113	147	94
Mean	5.47	8.40	5.91
Median	4.52	7.25	5.06
95% Range	2-14	3-20	2-15

Limitations

- The reagents supplied in this kit are optimized to measure hPRL levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Hemolyzed and lipemic specimens may give false values and should not be used.
- The KIT has no “high-dose hook” effect with hPRL levels up to 2500 ng/mL. Samples expected to have concentrations greater than the highest standard should be diluted with the S1 (0 ng/mL) and reassayed.
- The results of this assay should be used in conjunction with other pertinent clinical information.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer, the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information: Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 68 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	2 8°C	Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material
REF	Catalogue number		

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Updated: March/2021