RK-552A120901

rLH [^{125}I] RIA KIT

(REF: RK-552)

For Research Use Only. Not for use in diagnostic procedures.

The [^{125}I]rLH RIA system provides direct quantitative *in vitro* determination of Rat Luteinizing Hormone in plasma, tissue and cell culture. rLH can be assayed in the range of 0.8-50 ng/ml. Each kit contains materials sufficient for 120 determinations permitting the construction of one standard curve and the assay of 50 unknowns in duplicate.

Introduction

Rat luteinizing hormone (rLH) is a glycoprotein of molecular weight approximately 36 000 comprising two associated subunits (alpha and beta).

Both LH and follicle stimulating hormone (FSH) are termed gonadotrophins. These hormones are secreted by the basophilic cells of the anterior pituitary called gonadotrophs.

LH acts in females to induce ovulation and then maintain the secretory functions of the corpus luteum. In males, luteinizing hormone acts by stimulating the Leydig cells of the testes to produce testosterone.

The secretion of LH and FSH is regulated by a single gonadotrophin releasing hormone (GnRH, often referred to as luteinizing hormone releasing hormone, LHRH), a decapeptide produced in the hypothalamus. In turn, the secretions of the hypothalamic-pituitary unit are regulated by a negative feedback mechanism involving steroid hormones, inhibin and gonadotrophins.

Double antibody radioimmunoassays for rat LH have been developed. Estimates of the concentrations of LH in sera and serum extracts obtained by RIA have been lower than, but proportianal to, estimates obtained by bioassay. Measurement of circulating levels of LH is an important part of the investigation of disorders or perturbations of the hypothalamic/ pituitary/gonadal axis.

The role of the adrenal gland in the regulation of LH, FSH and prolactin secretion in lactating rats has recently been investigated. The pituitary-adrenal system seems capable of influencing the maintenance of normal secretion of gonadotrophins and prolactin as well as the maintenance of ovariun function during lactation in the rat.

Principle of method

This assay is based on the competition between unlabelled rLH and a fixed quantity of [^{125}I] – labelled rLH for a limited number of binding sites on a specific antibody. With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound will be inversely proportional to the concentration of added non-radioactive ligand.

The antibody bound rLH is then reacted with the separating second antibody reagent. Separation of the antibody bound fraction is effected by either magnetic separation or centrifugation of the separating reagent suspension and decantation of the supernatant.

Measurement of the radioactivity in the pellet enables the amount of labelled rLH in the bound fraction to be calculated. The concentration of unlabelled rLH in the sample is then determined by interpolation from a standard curve. The standard curve and samples should be prepared simultaneously.

Contents of the kit

1. 1 vial TRACER, lyophilized, reconstitution with 12.5 ml assay buffer, containing ~ 82 kBq , 2.2 µCi [^{125}I]rLH. The final solution contains [^{125}I]rLH in 0.025 M phosphate buffer pH7.5 containing 0.1%(w/v) sodium azide. Store at 2-8 °C.

2. 1 vial STANDARD, lyophilized, reconstitution with assay buffer, volume stated on the vial label. The final solution contains rLH at a concentration of 50 ng/ml. Store at 2-8 °C.

3. 1 vial ANTISERUM, lyophilized, reconstitution with 12.5 ml assay buffer. The final solution contains rabbit anti-rLH serum in 0.025 M phosphate buffer pH7.5 containing 0.1%(w/v) sodium azide. Store at 2-8 °C.

4. 1 vial ASSAY BUFFER concentrate (10.0 ml), dilution to 100 ml. On dilution this will give 0.025 M phosphate buffer, pH7.5, containing 0.1%(w/v) sodium azide. Store at 2-8 °C.

5. 1 vial SEPARATING SECOND ANTIBODY REAGENT (58 ml), ready for use, containing donkey anti-rabbit serum coated on to magnetisable polymer particles with sodium azide, colour-coded, blue-green. Store at 2-8 °C.

Pack leaflet

Materials, tools and equipment required

Pipettes or pipetting equipment with disposable tips (100µl, 200µl, 400µl, 500µl, 2.0ml and 12.5ml); disposable polypropylene or polystyrene tubes (12 x 75 mm); refrigerator; glass measuring cylinder (100ml); test tube rack; vortex mixer; plastic foil; separators, comprising magnetic base and assay rack - these are for use in the magnetic separation protocol; adsorbent tissue; gamma counter.

Note: For the centrifugal protocol, the following additional equipment will be required:

Decantation racks; refrigerated centrifuge capable of >1500 xg.

Specimen collection, storage

This section is provided for guidance only. It remains the investigator's responsibility to validate the chosen sample collection technique.

Blood samples

It is advised that if measurements are to be made on plasma samples, blood should be collected into tubes containing heparin (<20 units per ml of blood). Blood should be centrifuged immediately to remove cells and the plasma stored below -15°C prior to analysis. Serum samples can also be assayed with this kit. Samples may need to be diluted prior to assay depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

Tissue samples

Pituitary glands should be frozen immediately after removal and stored at -15°C to -30°C until required. Prior to analysis, glands should be homogenised or sonicated in phosphate buffered saline solution. Samples may need to be diluted depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

The efficiency of various extraction procedures for the estimation of rat anterior pituitary hormone content has recently been extensively examined .

Cell cultures

Cell culture media should be collected and stored below -15°C prior to analysis. Samples may need to be diluted depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

Preparation of reagents, storage

Storage: *see Contents of the kit*. At these temperatures each reagent is stable until expiry date. The actual expiry date is given on the package label and on the quality certificate.

Preparation: Equilibrate all reagents and samples to room temperature prior to use. Assay buffer: Warm the bottle containing assay buffer concentrate to 40°C or until the gel-like material melts. Temperatures above 60°C should be avoided. Transfer the contents of the bottle, with washings, to a 100 ml measuring cylinder and dilute to 100 ml with distilled or deionised water. Mix well. Assay buffer is used to reconstitute all other components.

The other components (except Separating reagent): Carefully add the required volume of assay buffer and replace stopper. Mix the contents of the bottles by inversion and swirling, taking care to avoid foaming.

Preparation of working standards

1. Label 6 polystyrene or polypropylene tubes 0.08, 0.16, 0.31, 0.62, 1.25 and 2.5.

2. Pipette 500 µl of assay buffer into all tubes.

3. Into the 2.5 tube pipette 500 µl of stock standard (50 ng/ml) and vortex thoroughly.

4. Transfer 500 µl from the 2.5 tube to the 1.25 tube and vortex thoroughly.

5. Repeat this doubling dilution successively with the remaining tubes.

6. 100 µl aliquots from each serial dilution give rise to 6 standard levels of rLH ranging from 0.08 ng to 2.5 ng/tube.

Note: One hundred microlitres (100 µl) of the reconstituted stock standard provided serves as the top standard (5 ng/tube). Working standards should be freshly prepared before each assay, and not re-used.

Assay procedure

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

1. Equilibrate all reagents to room temperature.
2. Prepare reagents and assay standards as described in the previous section.
3. Label 12x75 mm disposable tubes in duplicate for total count (TC), non-specific binding (NSB), zero standard (Bo), standards and samples.
4. Pipette **200 µl** assay buffer into NSB tubes and **100 µl** assay buffer into Bo tubes.

5. Starting with the most dilute, pipette **100** µl of each standard (S1-7) into the appropriately labelled tubes.
6. Pipette **100 µl** unknown sample (M_x) directly into appropriately labelled tubes.
7. Pipette **100 µl** antiserum into all tubes except NSB and TC.
8. Pipette **100 µl** of tracer into all tubes. The TC tubes should be stoppered and put aside for counting.
9. Vortex mix all tubes thoroughly. Cover the tubes, and incubate overnight (16-24 hours) at room temperature (15-30 °C).
10. Gently shake and swirl the bottle containing separating second antibody reagent (blue-green) to ensure a homogeneous suspension. Add **400 µl** into each tube except the TC. Vortex mix all tubes thoroughly and incubate at room temperature for 10 minutes.
11. Separate the antibody bound fraction using either magnetic separation or centrifugation as described below.

Magnetic separation

Attach the rack on the magnetic separator base and ensure that all tubes are in contact with the base plate. Leave for 15 minutes. After separation, do not remove the rack from the separator base. Pour off and discard the supernatant. Keeping the separator inverted, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

Centrifugation

Centrifuge all tubes at 4°C for 10 minutes at 1500 xg or greater. After centrifugation, place the tubes carefully into suitable decantation racks, then pour off and discard the supernatant. Keeping the tubes inverted, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

12. On completion of either magnetic or centrifugal separation, firmly blot the rims of the inverted tubes on the tissue pad to remove any adhering liquid. Do not re-invert the tubes once they have been turned upright.
13. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter.

Table 1. Assay Protocol, Pipetting Guide (all volumes are in microlitres)

Tubes	TC	NSB	Bo	Stan - dard	Sam- ple
Buffer	**-**	**200**	**100**	**-**	**-**
Stan- dard	-	**-**	**-**	**100**	**-**
Sample	**-**	**-**	**-**	**-**	**100**
Anti- serum	**-**	**-**	**100**	**100**	**100**
Tracer	**100**	**100**	**100**	**100**	**100**
Vortex mix and incubate for 16-24 hours at room temperature (15-30 °C).					
Separa- ting reagent	-	**400**	**400**	**400**	**400**
Vortex mix. Incubate for 10 minutes at room temperature.					
Separate either using magnetic separator for 15 minutes or by centrifugation for 10 minutes at >1500 xg .					
Decant tubes and blot on filter paper.					
Count radioactivity (60 sec/tube).					
Calculate the results.					

Calculation of results

Calculate the average count per minute (CPM) for each pair of assay tubes.
Calculate the percent NSB/TC using the following equation:

$$NSB/TC(\%) = \frac{NSB\ (cpm)}{TC\ (cpm)} \times 100$$

If the counter background is high, it should be subtracted from all counts.

Users may wish to subtract the average NSB cpm from all tubes except TC. If so the appropriate correction should be made. Calculate the percent Bo/TC using the following equation:

$$Bo/TC(\%) = \frac{Bo\ (cpm) - NSB\ (cpm)}{TC\ (cpm)} \times 100$$

Calculate the percent bound for each standard and sample using the following equation:

$$B/Bo(\%) = \frac{S1\text{-}7/M_x\ (cpm) - NSB\ (cpm)}{Bo\ (cpm) - NSB\ (cpm)} \times 100$$

A standard curve can be generated by plotting the percent B/Bo as a function of the log rLH concentration.
Plot B/Bo(%) (y-axis) against concentration ng standard per tube (x-axis). The concentration (ng per tube) value of the samples can be read directly from the graph (see Figure 1).

Table 2. Typical assay data

Tube	Conc. ng/tube	Mean counts (cpm)	B/TC (%)	B/Bo (%)
TC	-	31867	-	-
NSB	-	1021	3.2	-
Bo	-	12936	37.4	-
S1	0.08	12552	-	96.8
S2	0.16	12045	-	92.5
S3	0.31	10800	-	82.1
S4	0.62	8734	-	64.7
S5	1.25	6416	-	45.3
S6	2.5	4169	-	26.4
S7	5	2853	-	15.4

Note: The counts were obtained using new tracer. The counts will decline in line with the age of the tracer.
A decrease in Bo values will be observed during the shelf-life of this product. However, this drop in binding does not in our experience affect the curve parameters.

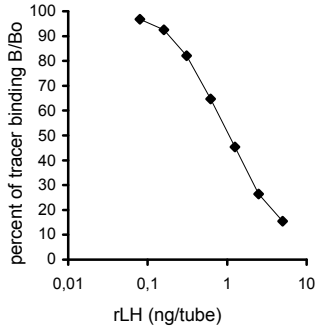


Figure 1: A typical standard curve
(*Do not use to calculate unknown samples!)*

Characterization of assay

Stability

The components of this assay system will have a shelf-life of at least 2 weeks from the date of despatch.
Upon arrival, all components should be stored at 2–8°C where they are stable until the expiry date printed on the pack label. Once reconstituted,all reagents should be stored at 2-8 °C where they are stable for at least 14 days.

Non-specific binding

The non-specific binding (NSB) defined as the proportion of tracer bound in the absence of antibody was determined to be <5%.
The NSB was independent of tracer batch and did not change over a 10-week storage period.

Sensitivity

The sensitivity, defined as the amount of rLH needed to reduce zero dose binding by two standard deviations was 0.09 ng/tube.

Specificity

The cross-reactivity, as determined by the concentration giving 50% B/Bo with a number of related compounds is shown below.

Compound	Cross-reactivity (%)
rat LH	100
rat LH (NIH-RP2)	119
rat LH (NIH-RP3)	143
rat TSH	0.56
rat FSH	<0.016
rat GH (NIH-RP2)	0.1
rat PRL	<0.08
rat ACTH	<0.0092

Reproducibility

The intra-assay precision for duplicate determinations was calculated by repeatedly measuring an unknown in the assay. The result obtained was 0.47 ± 0.03 ng, CV = 6.5%. The between assay precision was assessed by repeated measurement of the same samples in successive assays using different batches of reagents and different operators. These results are shown below:

Sample	Number of replicates	Mean ± 2SD	CV (%)
1	14	0.13±0.01	7.7
2	14	0.45±0.03	6.6
3	14	1.47±0.16	10.9

Dilutions of unextracted plasma and serum gave good parallelism with the standard curve (results from 2 experiments). The inter-assay precision values are shown below:

Sample volume (µl)	plasma	
	Measured conc. (ng/tube)	Calculated conc. (ng/tube)
25	0.08	3.20
50	0.15	3.00
100	0.31	3.10
Sample volume (µl)	serum	
	Measured conc. (ng/tube)	Calculated conc. (ng/tube)
25	0.10	4.00
50	0.19	3.80
100	0.41	4.10

Good recovery was obtained when rLH was added to plasma and serum:

Added conc. (ng/ml)	plasma		
	Expected conc. (ng/tube)	Measured conc. (ng/tube)	Recovery (%)
0	-	0.24	100
0.5	0.74	0.78	105
1.0	1.24	1.23	99
1.25	1.49	1.22	82

Added conc. (ng/ml)	serum		
	Expected conc. (ng/tube)	Measured conc. (ng/tube)	Recovery (%)
0	-	0.63	100
0.5	1.13	1.24	110
1.0	1.63	1.55	95
1.25	-	-	-

Additional information

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

Precautions

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.

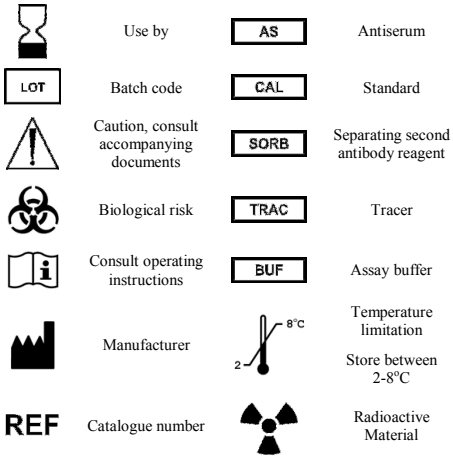
Chemical hazard

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 158 mg.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Safety data sheet

Product name:
Sodium azide
CAS No. 26628-22-8
R: 22-32 Toxic if swallowed. Contact with acids liberates very toxic gas.
S: (1/2)-28-45 (Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).
Composition:
Sodium azide solution.
Hazards identification:
Toxic if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.
First aid measures:
In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.
Fire fighting measures:
Dry chemical powder. Do not use water.
Accidental release:
Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.
Handling and storage:
Wear suitable protective clothing including overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.
Personal protection:
See above instructions for handling and storage.
Physical and chemical properties:
Formula weight: 65.01. Density: 1.850.
Stability and reactivity:
Avoid contact with metals and acid chlorides. This yields a very toxic gas.
Toxicological information:
LD50: 27 mg/kg oral, rat
LD50: 20 mg/kg skin, rabbit
Ecological information:
Not applicable
Disposal consideration:
Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.
Note: Inorganic azides will react with lead and copper plumbing fixtures to give explosive residues. Disposal of significant quantities of azides via such plumbing is not recommended.
Transport information :
No special considerations applicable.
Regulatory information:
The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.



Website: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

IZOTOP
INSTITUTE OF ISOTOPES CO. LTD.
1535 Budapest. Pf.: 851.
Tel.: (36-1)392-2577, Fax: (36-1)395-9247