Human Prolactin（PRL） ELISA Test KiT

NAME AND INTENDED USE

Detection Kit for Human Prolactin（PRL） (Enzyme-Linked ImmunoSorbent Assay , ELISA).It is used in quantitative tests for pituitary prolactin in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Human pituitary prolactin (PRL) is a 198 aminoacid protein produced by the lactotrophs of the anterior pituitary gland. A small proportion of pituitary PRL is glycosylated. PRL is secreted in an episodic mode, secretion is controlled negatively by dopamine. Due to the pulsatile mode of PRL secretion and its susceptibility to stress, mild to moderate hyperprolactinemia necessitates a double check to confirm that PRL level is truly abnormal. When this is confirmed, one should investigates various and frequent causes of moderately abnormal PRL, such as pregnangy, untreated hypothyroidism, chronic renal failure and cirrhosis. Many widely used drugs create hyperprolactinemia: among them, chlorpromazin, fluphenazin, haloperidol, metoclopramide, sulpiride, methyldopa, reserpine, verapramil, opiates, cimetidine and not systematically, estrogens. Neuro-hypothalamic diseases can also be responsible for mild to moderate hyperprolactinemia: tumors, cysts, metastasis, infiltrative diseases (sarcoidosis, tuberculosis, histiocytosis X), brain atrophy either senile or secondary to brain damage due to anoxia. Whatever the etiology, hyperprolactinemia can produce gonadal dysfunction in both sexes through impairment of gonadotropin secretions.

Significant hyperprolactinemia justifies immediate investigation to determine weither it is a secondary phenomenon or due primarily to a PRL secreting pituitary tumor.

Prolactinoma is the most common type of pituitary adenoma. It can be either sporadic or belong to a multiple endocrine neoplasia syndrome, type 1. The most frequent clinical features are hypogonadism and infertility. Women experience irregularities in menstrual cycle, anovulation and finally amenorrhea. Galactorrhea is present in up to 60% of these patients but may last for a short period of time in the clinical history. The other features of estrogen deficiency are also present. Men suffer from partial or total hypogonadism and/or infertility. Gynecomastia and galactorrhea occur also frequently, in up to 30% of men with massive hyperprolactinemia .

Mixed pituitary adenoma, secreting GH and PRL, GH-TSH and PRL, ACTH and PRL have also been described. Clinical pictures are dominated by the leading dyssecretion but mixed clinical situations can be observed.

PRINCIPLE

PRL uses a “sandwich principle”, Enzyme-linked immunological sorbent assay. To measure PRL levels in serum, plastic wells coated with a monoclonal antibody of PRL are supplied in the kit. After the patient’s specimen and another mono-antibody labelled with HRP are added, PRL, if present,is fixed to the solid phase antibody and creating a HRP- antibody PRL—antibody “sandwich”. After TMB substrate added, the result is obtained by EIA plate reader.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the PRL procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens can be stored up to 48 hours at 2~8°C.

Specimens held for a longer time can be frozen at –20°C and avoid repeated freeze melting. Serum samples with concentrations expected to be greater than 2000uIU/ml should be diluted with normal saline.

NOTE: If needed, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8×12 wells), Ready to use. Coated with anti-PRL antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2~8°C until expiration date.
2. HRP Conjugate: 1 vial of 6ml, Ready to use. Store at 2~8°C until expiration date.
3. Calibrator: 6 vials of 0.5 ml. Labeled with S0 to S5 and the concentration according to the label.
4. Control: 2 vials of 0.5ml. the concentration according to the label.
5. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2~8°C until expiration date.
6. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2~8°C until expiration date.
7. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2~8°C until expiration date.
8. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2~8°C until expiration date.
9. Plate sealer: 2 pieces.
10. Plastic resealable bag: 1 set.
11. Instruction manual: 1 copy.

RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 3 times with about 0.3ml diluted washing solution. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all water out and block the rim of well on absorbent paper for a few seconds. Filling each well with diluted washing solution and remain 10 seconds. Repeat these steps 3 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Storage. The whole kit should be stored at 2~8°C for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.
5. Read Procedure. Determine the absorbance (OD) of each well at 450nm with a microtiter plate reader. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
6. Control serum is prepared with human serum, which is tested negative of HBV, HCV and HIV. But it should still be considered as capable of transmitting viral diseases.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash buffer by diluting Wash Concentrate 20-fold with deionized water. The diluted washing solution is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. All the Calibrators and controls should set duplicate.
2. Dispense 50µl of calibrators / controls / samples into respective wells.
3. Dispense 50µl of HRP Conjugate to each well
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 1 hour.
5. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
6. Dispense 50µl of chromogen A to each well.
7. Dispense 50µl of chromogen B to each well.
8. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
9. Dispense 50µl of stop solution to each well and mix completely.
10. Read the absorbance of the plate within 10 minutes. (See read procedure)

CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation: log OD=B*log [concentration]+A

PERFORMANCE CHARACTERISTICS

1. Expected value

The cut-off value of PRL concentration for healthy subjects is:

Male: 2.0~17.0 ng/ml

Famale: 3.0~25.0 ng/ml

2. SensitivityThe detection limit of the assay is approximately 2.35 ng/ml
3. Precision

Interassay≤15%

Intraassay≤15%

4. Specificity: the cross reaction with TSH, HCG, LH, FSH, GH meets the requirements.

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