**FSH FOLLICLE STIMULATING HORMONE ELISA**

**Intended Use:** The Quantitative Determination of Follicle Stimulating Hormone Concentration in Human Serum by a Microplate Immunoassay assay (JENIA/ELISA).

**Summary And Explanation Of The Test:**
Follicle stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,000 daltons. The subunit is similar to other pituitary hormones [luteinizing hormone (LH) and thyroid stimulating hormone (TSH)] while the β-subunit is unique. The subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRHa) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to sites of action, the ovaries or testes.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis.

In women, FSH acts on the granulosa cells of the ovary, stimulating oogenesis. All ovariary menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases, near the time of ovulation occur, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level. The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic-pituitary—gonadal axis has been well established (1,2). In this method, FSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of FSH) are added and the reactants mixed. Reaction between the various FSH antibodies and native FSH forms a sandwich complex that binds with the streptavidin coated to this well. After the completion of the required incubation period, the enzyme-Follicle Stimulating hormone antibody bound conjugate (enzyme and non-enzyme conjugate, stimulation antibody conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

**Materials Provided:**
- FSH Calibrators
- FSH Enzyme Reagent
- Streptavidin Coated Microplate
- Working substrate Solution
- Stop Solution
- Product Insert

**Principal:**
**Immunoenzymometric assay:**
The essential reagents required for an immunoassay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition. In excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin covalently linked on the plate and exogenously added biotinylated monoclonal anti-FSH antibody.

Upon mixing, monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen on the antibody and antibody competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

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performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations. 

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens. 

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1. 

2. Plot the absorbance for each duplicate serum in order of increasing concentrations. The data are presented in Figure 1 for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

3. Draw the best-fit curve through the plotted points. 

To determine the concentration of FSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.214) intersects the dose response curve at 43.2 mIU/ml FSH concentration (See Figure 1).

EXAMPLE 1

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. The data presented in Figure 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

G. Parameters: 

In order to resolve assay results to be considered valid the following criteria should be met: 

1. The absorbance (OD) of calibrator F should be $> 1.25$. 

2. Four out of six control pools should be within the established ranges.

3. The absorbance of the duplicate wells should be within 10% of each other.

4. The absorbance of the calibrator should be consistent.

5. The absorbance of the calibrator should be consistent throughout the assay.

6. The absorbance of the calibrator should be consistent with the reference method.

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K. Roitt I, Schwartz S, Wick G 1996 Immunoochemical mapping of