REFERENCES:
1. Qiu Q; Kuo A; Todd H; Dias JA; Gould JE; Overstreet JW; Lasley BL. Enzyme immunoassay method for total urinary follicle-stimulating hormone (FSH) beta subunit and its application for measurement of total urinary FSH. Fertil Steril 1998; 69(2):278-85.
6. Popovic V; Micic D; Damjanovic S; Calovic L; Rolovic Z; Mijovic A; Petakov M; Manojlovic D; Micic J. Further evidence for differential regulation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) increased FSH and decreased LH levels in a patient with familial pure gonadal dysgenesis. Postgrad Med J 1992; 68(805):925-7.

Follicle Stimulating Hormone (FSH) SA ELISA
Catalog No.: IB19103 (96 Tests)

INTENDED USE
The FSH ELISA Kit is intended for the measurement of FSH in human serum. For research use only – Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION
Follicle-Stimulating Hormone (FSH) is a glycoprotein produced by the anterior pituitary gland. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally; therefore the biological and immunological properties of each are dependent on the unique beta subunit. In the female, FSH stimulates folliculogenesis, prepares ovarian follicles for action by LH and enhances the LH induced release of estrogen. FSH levels are elevated after menopause, castration and in premature ovarian failure. Although there are significant exceptions, ovarian failure is indicated when random FSH concentrations exceeds 40 mIU/ml. In the male, FSH stimulates seminiferous tubule and testicular growth and is involved in the early stages of spermatogenesis. Oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations, but levels of LH are elevated. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.

PRINCIPLE OF THE TEST
The FSH ELISA kit is a solid phase assay using streptavidin/biotin method. The samples and Anti-FSH/Anti-Biotin conjugate are added to the wells coated with Streptavidin. FSH in the serum forms a sandwich between specific antibodies labeled with biotin and HRP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of FSH in the samples. A standard curve is prepared relating color intensity to the concentration of the FSH.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with Streptavidin</td>
<td>12x8x1</td>
</tr>
<tr>
<td>FSH Standard</td>
<td>6 vials (ready to use)</td>
</tr>
<tr>
<td>FSH Enzyme Conjugate</td>
<td>1 bottle (ready to use)</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>1 bottle (ready to use)</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 bottle (ready to use)</td>
</tr>
<tr>
<td>20X Wash Concentrate</td>
<td>1 bottle</td>
</tr>
</tbody>
</table>

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY
1. Store the kit at 2 - 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.

Manufactured For:
Immuno-Biological Laboratories, Inc. (IBL-America)
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Minneapolis, MN 55432
Tel: 763-780-2955 / Fax: 763-780-2968
Email: info@ibl-america.com
www.ibl-america.com

2014-07-07
WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The Standard contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories.” 1984

2. This kit is for research use only.

3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which samples or kit reagents are handled.

4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. It is recommended that standards, control and serum samples be run in duplicate.

6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SAMPLE COLLECTION HANDLING
1. Collect blood samples and separate the serum immediately.

2. Samples may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.

3. Avoid multiple freeze-thaw cycles.

4. Prior to assay, frozen sera should be completely thawed and mixed well.

5. Do not use grossly lipemic samples.

REAGENTS PREPARATION
20X Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

ASSAY PROCEDURE
Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.

2. Pipette 50 μl of FSH standards, control and sera into selected wells.

3. Add 100 μl of enzyme conjugate to all wells.

4. Cover the plate and incubate for 60 minutes at room temperature (18-26°C).

5. Remove liquid from all wells. Wash wells three times with 300 μl of 1X wash buffer. Blot on absorbent paper towels.

6. Add 100 μl of TMB substrate to all wells.

7. Incubate for 15 minutes at room temperature.

8. Add 50 μl of stop solution to all wells. Shake the plate gently to mix the solution.

9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS
The standard curve is constructed as follows:

1. Check FSH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

2. To construct the standard curve, plot the absorbance for the FSH standards (vertical axis) versus the FSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

EXPECTED VALUES
It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

<table>
<thead>
<tr>
<th>Classification</th>
<th>Normal Range (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.0-15</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Follicular/Luteal phase</td>
<td>2.0-10</td>
</tr>
<tr>
<td>Mid-cycle</td>
<td>2.0-20</td>
</tr>
<tr>
<td>Pregnant</td>
<td>Less than 2.0</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>Greater than 15</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE TEST
1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Example of a Standard Curve

<table>
<thead>
<tr>
<th>Conc. mIU/mL</th>
<th>OD 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>0</td>
</tr>
<tr>
<td>Std 2</td>
<td>5</td>
</tr>
<tr>
<td>Std 3</td>
<td>10</td>
</tr>
<tr>
<td>Std 4</td>
<td>25</td>
</tr>
<tr>
<td>Std 5</td>
<td>50</td>
</tr>
<tr>
<td>Std 6</td>
<td>100</td>
</tr>
</tbody>
</table>