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Establishment of a Double-Antibody Sandwich Enzyme Immunoassay for Determination of FSH in Human Serum

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Introduction

Follicle stimulating hormone (FSH) is a glycoprotein gonadotropin synthesized and secreted by basophilic cells in the anterior pituitary gland, and it plays a crucial role in regulating the growth of reproductive cells and hormone synthesis, contributing significantly to reproductive activities. In order to accurately quantify FSH levels in human serum for biomarker analysis, an enzyme-linked immunosorbent assay (ELISA) was developed and qualified in this study.

Methods

In this study, a sandwich immunoassay was developed for quantitative determination of FSH biomarkers in human serum specimens. A mouse anti-human FSH monoclonal antibody (capture reagent) is directly coated on 96-well microplate. Samples are then added to the microplate and the FSH in the samples bind to the capture antibodies. Subsequently, a biotin-labelled mouse anti-human FSH antibody (different antibody from the capture reagent) is added as the detection reagent to form a bridge structure. This is followed by the addition of ploy-SA-HRP and TMB for detection. The assay design is illustrated below.

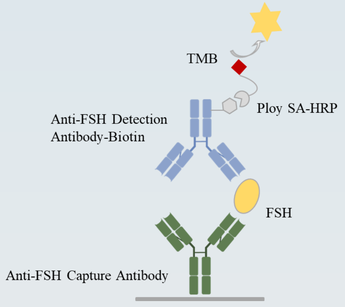

Figure 1. Assay design

Assay Results

Dynamic Range

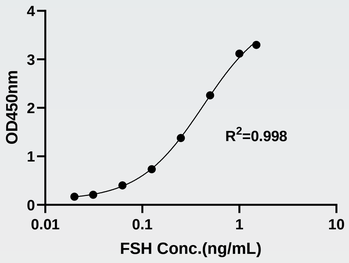

Figure 2. Representative standard curve

Table 1. Standard Curve Summary of Analytical Runs

Run Number	Test Result for STD of FSH (ng/mL)						
	1.50	1.00	0.50	0.25	0.13	0.06	0.03
1	1.49	1.03	0.50	0.24	0.12	0.07	0.03
2	1.35	1.09	0.56	0.22	0.12	0.06	0.03
3	1.35	1.07	0.54	0.24	0.12	0.06	0.03
4	1.33	1.07	0.55	0.24	0.12	0.07	0.03
5	1.36	1.09	0.50	0.25	0.12	0.07	0.03
6	1.35	1.11	0.53	0.24	0.12	0.06	0.04
Mean Conc.	1.37	1.08	0.53	0.24	0.12	0.07	0.03
%CV	4.3	2.4	4.5	3.4	1.6	4.6	6.1
%RE	-8.6	7.8	6.2	-5.2	-1.6	4.0	5.6
Total Error (%)	12.9	10.2	10.7	8.6	3.2	8.6	11.7

The dynamic range is 0.03 ~ 1.50 ng/mL (corresponding to 0.54 ~ 26.25 mIU/mL), and 0.02 ng/mL is anchor point.

Accuracy and Precision (A&P)

The analytical method was performed in 6 A&P runs over 3 days by using 5 levels of QC samples to assess precision and accuracy. The experimental results are as follows:

Table 2. Validation results of accuracy and precision

QCs	Nominal (ng/mL)	Back-calc.MeanConc. (ng/mL)	%CV	%RE	Total Error%
ULOQ	1.50	1.34	8.7	-10.5	19.3
HQC	1.00	1.04	8.5	4.2	12.7
MQC	0.40	0.40	9.9	0.8	10.7
LQC	0.10	0.10	8.2	-2.0	10.2
LLOQ	0.03	0.03	9.4	2.4	11.8

Parallelism

Three individual serum samples were diluted for parallelism assessment. As shown in Table 3, each sample showed parallelism upon being diluted into the assay range. The results demonstrates immunological similarity of the standard to the endogenous analyte and the validity of a surrogate matrix.

Table 3. Validation results of parallelism

Sample	Dulition Factor	Mean OD450nm	Measured MeanConc. (ng/mL)	%CV	Corrected Conc.(ng/mL)	Recovery (%)
1	1	0.89	0.36	1.8	0.36	N/A
	2	0.60	0.22	0.2	0.44	N/A
	4	0.39	0.13	0.0	0.52	100%
	8	0.24	0.06	4.9	0.48	92%
	16	0.16	＜LLOQ	N/A	N/A	N/A
2	1	1.64	0.82	1.2	0.82	N/A
	2	1.08	0.46	5.1	0.92	N/A
	4	0.75	0.29	5.7	1.16	100%
	8	0.47	0.16	6.1	1.28	110%
	16	0.26	0.07	7.4	1.12	97%
3	1	2.64	0.81	4.8	0.81	N/A
	2	2.09	0.50	0.1	0.99	N/A
	4	1.62	0.32	3.2	1.29	100%
	8	1.01	0.17	4.5	1.32	102%
	16	0.56	0.08	7.8	1.23	96%

Selectivity

Selectivity was evaluated by spiking a certain level of FSH standard into 10 individuals human serum samples. As shown in Table 4, all test sample passed and there is no matrix effect.

Table 4. Validation results of selectivity

Sample	Endogenous Conc. (ng/mL)	Spiked Conc. (ng/mL)	Measured Conc. (ng/mL)	Recovered Conc. (ng/mL)	Spiked %AR
1	0.45	0.10	0.55	0.45	100%
2	0.47	0.10	0.58	0.48	102%
3	0.11	0.10	0.23	0.13	118%
4	0.10	0.10	0.22	0.12	120%
5	0.12	0.10	0.21	0.11	92%
6	0.05	0.10	0.14	0.04	80%
7	0.20	0.10	0.32	0.22	110%
8	0.21	0.10	0.27	0.17	81%
9	0.13	0.10	0.23	0.13	100%
10	0.12	0.10	0.22	0.12	100%

Specificity

To assess the specificity of this assay, the QC samples were spiked with recombinant hCG, an irrelevant hormone that could be present in a test sample. As shown in Table 5, there was no interference, indicating that the assay is specific to FSH.

Table 5. Validation results of specificity

hCG Conc.(ng/mL)	Spiked FSH Conc. (ng/mL)	Measured MeanConc. (ng/mL)	%CV	%RE
200.00	1.00	1.04	0.2	3.6
100.00		0.98	1.6	-1.7
50.00		1.04	14.9	4.3
25.00		1.07	1.0	6.8
12.50		1.04	0.2	4.5

Stability

To evaluate the sample stability, we treated QC samples under the following conditions: up to 24 hours at room temperature (RT), 3 days at 2 ~ 8℃, 7 days at 2 ~ 8℃, and 5 freeze-thaw cycles (F/T). As shown in Table 6, all samples were demonstrated to be stable under all test conditions.

Table 6. Validation results of stability

Condition	Sample	Nominal (ng/mL)	Measured MeanConc. (ng/mL)	%CV	%RE
24h hours at RT	HQC	1.00	1.06	6.7	5.6
	LQC	0.10	0.10	0.3	-3.2
3 days 2~8℃	HQC	1.00	1.05	0.3	5.1
	LQC	0.10	0.11	0.1	6.0
7 days 2~8℃	HQC	1.00	0.95	1.5	-5.0
	LQC	0.10	0.11	2.0	4.8
5 F/T	HQC	1.00	1.00	1.5	-0.5
	LQC	0.10	0.10	0.2	2.8

Conclusion

In this study, an ELISA was established for the determination of FSH in human serum. The assay demonstrated the quantification range of 0.03 ~ 1.50 ng/mL (0.54 ~ 26.25 mIU/mL), with acceptable precision, accuracy, selectivity, parallelism, specificity and short-term stability of the samples. The assay is suitable for FSH biomarker sample analysis.