

CaptureSelect™ FSH Ligand Leakage ELISA

INSTRUCTIONS

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Kit contents

Note: After thawing and before use, spin the tubes to ensure that all reagents are at the bottom of the tube.

Item	Description	Storage
Coating reagent (green label)	Goat IgG anti-FSH affinity ligand, 100 µL	-20°C (-4°F)
Standard solution (blue label)	CaptureSelect™ FSH affinity ligand, 100 µL	
Biotinylated reagent (yellow label)	Biotinylated Goat IgG anti-FSH affinity ligand, 100 µL	

Introduction

The CaptureSelect™ FSH Ligand Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of less than 1 ng/mL of FSH affinity ligand that may be present in product purified with CaptureSelect™ Follicle Stimulating Hormone affinity media, which contains the FSH affinity ligand as capturing agent. The assay is designed to minimize interference and to provide accurate quantitation in the presence of human FSH. The FSH Ligand Leakage ELISA can be used as a tool to aid in optimal purification process development and in routine quality control of in-process streams as well as final product.

Principle of the assay

The CaptureSelect™ leakage assays enable detection of the affinity ligand in solutions with and without the presence of the target protein. These sandwich assays involve the following steps:

- A microtiter plate is coated with affinity-purified anti-affinity ligand.
- Samples containing the affinity ligand are incubated in the coated plate wells.
- Bound affinity ligand is detected by biotinylated affinity ligand.
- Streptavidin horseradish peroxidase conjugate is added to bind to the biotinylated antibody in the sandwich complex.
- Substrate reactive with horseradish peroxidase (tetramethylbenzidine-hydrogen peroxide) is added.
- The amount of hydrolyzed substrate is determined and is directly proportional to the concentration of affinity ligand present.

Required materials and equipment (not provided)

- PBS: Phosphate buffered saline pH 7.4
- PBST: Phosphate buffered saline (PBS) pH 7.4 + 0.05 (v/v)% Tween 20 Solution
- Bovine Serum Albumin (BSA), Fraction V 99% pure (Sigma-Aldrich A3059)
Note: Other qualities of Bovine Serum Albumin or other blocking proteins might result in higher background levels.
- Dilution Buffer A:
 - Dilution Buffer A: 0.05 (v/v)% Tween® 20 Solution in PBS pH 7.4
 - 2X Dilution Buffer A: 0.1 (v/v)% Tween® 20 Solution in PBS pH 7.4
- Blocking solution: 4 (w/v)% BSA in PBS pH 7.4
- Streptavidin-Horseradish Peroxidase diluted immediately before using according to manufacturer guidelines
- Tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) substrate (prepare 1:1 solution immediately before use)
- 1 M H₂SO₄
- Microtiter plate (Maxisorp, Nunc)
- Microtiter plate shaker
- Microtiter plate reader (450 nm)
- Milli-Q® water

Procedure

Coat the plate

1. Make a 1:100 dilution of the Coating reagent with PBS pH 7.4.
2. Add 100 μ L diluted Coating reagent to each well in a microtiter plate and incubate overnight at 4°C (39°F). This step captures fragments of the leached ligand.

Prepare standards

1. Prepare a 6.4 μ g/mL stock Standard dilution series: Add 10 μ L Standard solution to 770 μ L Dilution Buffer A.
2. Using the stock Standard solution from step 1, prepare a standard dilution series according to the table below.

Tube	Concentration (ng/mL)	Standard	Dilution Buffer A
1	64.0	10 μ L diluted Standard solution	990 μ L
2	16.0	250 μ L 64.0 ng/mL	750 μ L
3	8.0	500 μ L 16.0 ng/mL	500 μ L
4	4.0	500 μ L 8.0 ng/mL	500 μ L
5	2.0	500 μ L 4.0 ng/mL	500 μ L
6	1.0	500 μ L 2.0 ng/mL	500 μ L
7	0.5	500 μ L 1.0 ng/mL	500 μ L
8	0.25	500 μ L 0.5 ng/mL	500 μ L
9	0	0	500 μ L

Prepare assay samples

Dilute 75 μ L sample with 75 μ L of 2X Dilution Buffer A.

ELISA assay procedure

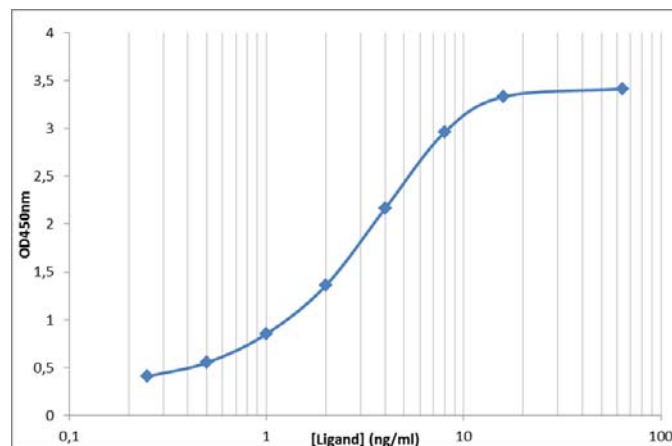
1. Block the plate:
 - a. Wash the coated plate 5 times with PBST.
 - b. Add 250 μ L/well of Blocking solution to the coated plate. Leave at room temperature for 30 minutes on a microtiter plate shaker.
 - c. Wash the plate 1 time with PBST.
2. Add samples and standards:
 - a. Add 100 μ L of each concentration of the standard dilution series (0 to 64.0 ng/mL) or sample to appropriate wells.
 - b. Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
 - c. Wash the plate 5 times with PBST.
3. Add Biotinylated reagents (detects leached ligand):
 - a. Make a 1:100 dilution of the Biotinylated reagents with Dilution Buffer A.
 - b. Add 100 μ L diluted Biotinylated reagents to each well containing sample or standard and incubate the plate 1 hour at room temperature.
 - c. Wash the plate 5 times with PBST.

4. Add Streptavidin-Horseradish peroxidase (colorimetric reagent that binds to the biotinylated reagents):
 - a. Dilute in Dilution Buffer A according to the manufacturer's guidelines.
 - b. Add 100 μ L diluted Streptavidin-Horseradish peroxidase to each well containing sample or standard.
 - c. Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
 - d. Wash the plate 5 times with PBST.
 - e. Wash the plate 2 times with Milli-Q® water.
5. Develop and read the plate:
 - a. Prepare a 1:1 solution of TMB:H₂O₂ substrate.
 - b. Add 100 μ L to each well containing sample or standard.
 - c. Incubate the plate for approximately 25 minutes on a microtiter plate shaker.
 - d. When the background signal starts to develop, add 50 μ L 1 M H₂SO₄ to stop the coloring reaction and achieve a maximal signal-to-noise ratio.
 - e. Measure the OD of the microtiter plate at 450 nm with a microtiter plate reader.

Calculate results

Create a standard curve using the OD values from the standards reported in ng/mL. Use curve-fitting routines such as 4-parameter logistic fit. Do not use linear regression analysis to interpolate values for samples, which may lead to significant inaccuracies.

Figure 1 Example calibration curve CaptureSelect™ FSH Ligand Leakage ELISA.



Validate the assay

Perform validation studies that include at least the following experiments to validate this kit for your application: 1) Intra- and inter-assay precision experiments to establish reproducibility, 2) Recovery experiments using test samples with known amounts of the 500 μ g/mL Standard solution, which is included in the kit.

Ordering information

CaptureSelect™ FSH Ligand Leakage ELISA	Part Number
1 assay	810318001
10 assays	810318010

For more information

For more information on CaptureSelect™ products, go to www.lifetechnologies.com/captureselect

Limited Product Warranty

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Safety information

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

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