CaptureSelect™ FSH Ligand Leakage ELISA

Catalog Numbers 810318001 and 810318010

Pub. No. MAN0009642 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The CaptureSelect™ FSH Ligand Leakage ELISA is designed to detect possible leached ligand of follicle stimulating hormone purified using CaptureSelect™ FSH affinity matrix. The ELISA provides typical sensitivity and accurate quantitation of possible leached ligand greater than or equal to 1 ng/mL.

Contents and storage

Contents	Cat. No. 810318001 (1 assay)	Cat. No. 810318010 (10 assays)	Storage
Coating Reagent (green cap), Goat IgG anti-FSH affinity ligand	100 µL	1,000 µL	–20°C (–4°F)
Standard Solution (blue cap), CaptureSelect™ FSH affinity ligand	100 µL	1,000 µL	
Biotinylated Reagent (yellow cap), Biotinylated Goat IgG anti-FSH affinity ligand	100 µL	1,000 µL	

Principle of the assay

FSH affinity ligand that is immobilized on chromatography media can leach off the resin and co-elute with the target protein. The CaptureSelect™ FSH Ligand Leakage ELISA assay enables detection of the affinity ligand.

The assay is a sandwich-type ELISA that uses a horseradish peroxidase detection system and involves the following steps:

1. The FSH affinity ligand that is present in the samples and prepared standard dilution series is captured by affinity-purified anti-affinity ligand polyclonal goat antibodies coated on the plate.
2. The bound affinity ligand is detected by adding biotinylated affinity ligand.
3. Streptavidin-Horseradish Peroxidase conjugate is added to bind to the biotinylated antibody in the sandwich complex.
4. Substrate reactive with horseradish peroxidase [tetramethylbenzidine (TMB)] is added.
5. The amount of hydrolyzed substrate is determined and is directly proportional to the concentration of affinity ligand present.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com.

Catalog numbers that appear as links open the web pages for those products.

Critical materials

Critical materials have a significant impact on the assay performance. To reduce assay development and optimization efforts, recommendations for the critical materials are listed in Table 1.

Table 1 Critical materials

Item	Source
Blocking reagent	
StartingBlock™ (PBS) Blocking Buffer	37578
Blocker™ Casein in PBS	37528
Bovine Serum Albumin (BSA), Fraction V 99% pure	Sigma-Aldrich™ A3059
StabilCoat™ Immunoassay Stabilizer	SURMODICS SC01
Microtiter plate	
Nunc™ MaxiSorp™ flat-bottom	442404
Streptavidin-Horseradish Peroxidase conjugate	
HRP-Conjugated Streptavidin	N100
Streptavidin/HRP (Conjugate)	Agilent Dako p3097

Item	Source
Tetramethylbenzidine (TMB) substrate	
1-Step™ Ultra TMB-ELISA Substrate Solution	34028
TMB Substrate Solution	N301
ELISA TMB Stabilized Chromogen	SB02
BD OptEIA™ TMB Substrate Reagent Set	BD Biosciences 555214

Non-critical materials

Non-critical materials have limited to no impact on the assay performance. You can perform the assay with the non-critical materials you may already have.

- PBS: Phosphate buffered saline pH 7.4
- PBST: Phosphate buffered saline (PBS) pH 7.4 + 0.05 (v/v)% Tween™ 20 Solution
- Demineralized water
- 0.2M–1M H_2SO_4
- Pipettes, 10–100 µL
- Multichannel pipettes, 100 µL
- Microtiter plate reader spectrophotometer with wavelength capability at 450 nm
- Microtiter plate shaker
- Microcentrifuge tubes

Before you begin

IMPORTANT! The assay should be performed by technically qualified individuals.

- Read through the entire procedure.
- Bring all reagents to room temperature before use.
- Prepare other materials as needed (see “Required materials not supplied” on page 1).
- Prepare the standard dilution series according to the assay protocol.
- Set the plate reader to 450-nm wavelength.
- Locate the assay certificate (see “Locate the assay certificate” on page 2).

Locate the assay certificate

Each ELISA assay lot is performance tested, and the results are captured in an assay certificate. The assay certificate provides the reagent concentrations, internal control recovery, an example calibration curve, and the recommended TMB incubation time.

1. Go to **www.thermofisher.com**
2. In the search bar, enter ***certificate*** `<lot number>`, where `<lot number>` is the lot number for your ELISA assay (for example, 220822-101).

Procedural guidelines

- To achieve good assay reproducibility and sensitivity, you must wash the plate extensively after each step.
- Sample components (such as pH, salts, impurities, or the target protein itself) can affect the assay performance. The effects should be studied in order to yield acceptable background signals, sensitivity, and recovery. To correct for these components, add them to the standard dilution series in similar concentrations. Ensure that these additions do not contain leached ligand, which will affect the assay performance. If these additions are not sufficient, investigate the other sample preparation methods described in “Optional sample treatment protocols” on page 4.
- During optimization and validation of the leakage ELISA, the TMB incubation time is an important parameter to investigate. As a guideline for the TMB incubation time, you can use the time specified on the lot-specific assay certificate; however, small deviations may be required, based on your process.
- After the TMB incubation is stopped using the stop solution, measure the plate within 5 minutes. After 5 minutes, black precipitation can occur in the wells and affect the assay performance.
- To prevent systematic inaccuracies, maintain a constant timing sequence from well-to-well, especially when adding the TMB and stop solution.

Wash the plate

The ELISA plate can be washed using an automatic plate washer.

Alternatively, use the following manual procedure.

1. Empty the contents of the wells into a waste bin or sink.
2. Gently but firmly tap the plate upside down on a paper towel or other absorbent material to remove most of the residual liquid.

   Note: Tapping too hard may dislodge the antibody bound material from the plate and cause elevated background signals and overall lower signals.
3. Completely fill the wells with PBST using a squirt bottle or a pipette.

   Alternatively, fill a small tray or bowl with PBST, then immerse the plate completely to fill all wells.

Methods

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

Coat the plate

1. Make a 1:100 dilution of the Coating Reagent with PBS pH 7.4.
2. Add 100 µL of diluted Coating Reagent to each well in a microtiter plate.
3. Incubate the plate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature. Alternatively, incubate the plate overnight at 4°C (39°F), without shaking.

Prepare standards

1. Prepare a 6.4 µg/mL stock Standard Solution: Add components to a microcentrifuge tube according to Table 2.

Table 2 Stock Standard Solution

Component	Volume
Standard Solution (blue cap)	10 µL
PBST	770 µL
Total	**780 µL**

2. Using the stock Standard Solution from step 1, prepare a standard dilution series according to Table 3.

Table 3 Standard dilution series

Tube	Conc. (ng/mL)	Standard	PBST
1	64.0	10 µL of stock Standard Solution	990 µL
2	16.0	250 µL of 64.0 ng/mL	750 µL
3	8.0	500 µL of 16.0 ng/mL	500 µL
4	4.0	500 µL of 8.0 ng/mL	500 µL
5	2.0	500 µL of 4.0 ng/mL	500 µL
6	1.0	500 µL of 2.0 ng/mL	500 µL
7	0.5	500 µL of 1.0 ng/mL	500 µL
8	0.25	500 µL of 0.5 ng/mL	500 µL
9	0	0	500 µL

Prepare the samples

Dilute the sample by adding the following to a microcentrifuge tube:

Table 4 Sample dilution

Component	Volume
Sample	75 µL
PBST	75 µL
Total	**150 µL**

ELISA assay procedure

IMPORTANT! For all wash steps, wash the plate according to "Wash the plate" on page 2.

1. Block the plate:
   a. Wash the plate 1 time with PBST.
   b. Add 200 µL/well of Blocking solution to the coated plate.
   c. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 30 minutes at room temperature.

2. Add samples and standards:
   a. Wash the plate 1 time with PBST.
   b. Add 100 µL of each concentration of the standard dilution series (0–64.0 ng/mL) or sample to the appropriate wells.
   c. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature.

3. Add Biotinylated Reagent:
   a. Wash the plate 3 times with PBST.
   b. Make a 1:100 dilution of the Biotinylated Reagent with PBST.
   c. Add 100 µL of the diluted Biotinylated Reagent to each well.
   d. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature.

4. Add diluted Streptavidin-Horseradish Peroxidase conjugate:
   a. Wash the plate 3 times with PBST.
   b. Dilute the Streptavidin-Horseradish Peroxidase conjugate with PBST according to the manufacturer guidelines.
   c. Add 100 µL of the diluted peroxidase to each well.
   d. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature.

5. Develop and read the plate:
   a. Wash the plate 3 times with PBST.
   b. Wash the plate 2 times with demineralized water.
   c. Add 100 µL of TMB substrate to each well.
   d. Cover the plate, then incubate on a microtiter plate shaker for the recommended incubation time at room temperature.

      Note: The recommended incubation time is stated on the assay certificate. See "Locate the assay certificate" on page 2.
   e. Add 50 µL of 0.2–1 M H_2SO_4 to each well to stop the reaction.
   f. Within 5 minutes, measure the absorbance of the microtiter plate at 450 nm with a microtiter plate reader.

Calculate results

1. Construct a standard curve with values 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, because this method may lead to significant inaccuracies.

2. Use the standard curve and the sample absorbance to determine the ligand concentration in your sample.

Information about the calibration curve obtained during the quality assessment, including the reagents and incubation time used, is provided in the assay certificate. See "Locate the assay certificate" on page 2.

Validate the assay

Perform validation studies that include at least the following experiments to validate this assay for your application:

- Intra- and inter-assay precision experiments to establish reproducibility.
- Recovery experiments using test samples with known amounts of FSH affinity ligand. The test samples can be prepared from the 500 µg/mL Standard Solution that is included in the kit.

  Note: The test samples can be aliquoted and stored at -20°C for long-term storage.

Optional sample treatment protocols

Because every protein purified with the CaptureSelect™ affinity resins and purification process can be different, matrix effects on the FSH Ligand Leakage ELISA assay are difficult to predict. If correcting for the buffer matrix influences is not sufficient for obtaining good recoveries, you may need to perform one of the following sample treatment options.

Note: These sample treatment protocols have not been tested with the FSH Ligand Leakage ELISA assay.

Prepare the standards and samples: Heat treatment

Heat treatment is performed on the sample and standard dilution series to remove the target protein by precipitation while the ligand stays in solution, increasing assay performance and recovery.

1. Prepare a 6.4 µg/mL stock Standard Solution: Add components to a microcentrifuge tube according to Table 5.

   Table 5 Stock Standard Solution

Component	Volume
Standard Solution (blue cap)	10 µL
PBST	770 µL
Total	**780 µL**

2. Using the stock Standard Solution from step 1, prepare a 2X concentrated standard dilution series according to Table 6.

   Table 6 Standard dilution series

Tube	Conc. (ng/mL)	Standard	PBST
1	128.0	20 µL of stock Standard Solution	980 µL
2	32.0	250 µL of 64.0 ng/mL	750 µL
3	16.0	500 µL of 16.0 ng/mL	500 µL
4	8.0	500 µL of 8.0 ng/mL	500 µL
5	4.0	500 µL of 4.0 ng/mL	500 µL
6	2.0	500 µL of 2.0 ng/mL	500 µL
7	1.0	500 µL of 1.0 ng/mL	500 µL
8	0.5	500 µL of 0.5 ng/mL	500 µL
9	0	0	500 µL

3. Dilute the standards from the dilution series 1:1 with Dilution Buffer A.

   To prepare Dilution Buffer A: Combine PBST and target protein. Use target protein with a concentration comparable to the samples.

4. Dilute the samples 1:1 with PBST.

5. Incubate the standards and samples for 15 minutes at 95°C, then allow to cool to room temperature.

6. Centrifuge the standards and samples at 20,000 × *g* for 5 minutes.

7. Transfer the supernatant to a clean tube.

8. Proceed to “ELISA assay procedure” on page 3.

Prepare the standards and samples: DTT treatment

DTT is added to the sample and standard dilution series to reduce the ligand-target protein complex, increasing assay performance and recovery.

1. Prepare a 6.4 µg/mL stock Standard Solution: Add components to a microcentrifuge tube according to Table 7.

   Table 7 Stock Standard Solution

Component	Volume
Standard Solution (blue cap)	10 µL
PBST	770 µL
Total	**780 µL**

2. Using the stock Standard Solution from step 1, prepare a 2X concentrated standard dilution series according to Table 8.

   Table 8 Standard dilution series

Tube	Conc. (ng/mL)	Standard	PBST
1	128.0	20 µL of stock Standard Solution	980 µL
2	32.0	250 µL of 64.0 ng/mL	750 µL
3	16.0	500 µL of 16.0 ng/mL	500 µL
4	8.0	500 µL of 8.0 ng/mL	500 µL
5	4.0	500 µL of 4.0 ng/mL	500 µL
6	2.0	500 µL of 2.0 ng/mL	500 µL
7	1.0	500 µL of 1.0 ng/mL	500 µL
8	0.5	500 µL of 0.5 ng/mL	500 µL
9	0	0	500 µL

3. Dilute the standards from the dilution series 1:1 with Dilution Buffer A.

   To prepare Dilution Buffer A: Combine PBST, target protein, and 400 µM of DTT. Use target protein with a concentration comparable to the samples.

4. Dilute the samples 1:1 with Dilution Buffer B.

   To prepare Dilution Buffer B: Combine PBST and 400 µM of DTT.

5. Incubate the standards and samples for 1 hour at 60°C, then allow to cool to room temperature.

6. To ensure that no liquid is in the tube caps, centrifuge the standards and samples at 5,000 rpm for 30 seconds.

7. Proceed to “ELISA assay procedure” on page 3.

Limited product warranty

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0009642

Revision	Date	Description
B.0	11 July 2023	• Branding and trademarks were updated. • The list of required materials was updated. • Information about how to locate the assay certificate was added. • A procedure to wash the plates was added. • The ELISA assay procedure was updated. • Optional sample treatment protocols were added.
A.0	29 October 2013	New document.