


CaptureSelect™ TSH Ligand Leakage ELISA

Catalog Numbers 810356201 and 810356210

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 **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™ TSH Ligand Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of 1 ng/mL TSH affinity ligand that may be present in product purified with CaptureSelect™ TSH affinity media, which contains the TSH affinity ligand as the capturing agent. The TSH 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.

Contents and storage

Contents	Cat. No. 810356201 (1 assay)	Cat. No. 810356210 (10 assays)	Storage
Coating Reagent (green cap), Goat IgG anti-TSH affinity ligand	100 µL	1,000 µL	-20°C (-4°F)
Standard Solution (blue cap), TSH affinity ligand	100 µL	1,000 µL	
Biotinylated Reagent (yellow cap), Biotinylated Goat IgG anti-TSH affinity ligand	100 µL	1,000 µL	

Principle of the assay

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

1. A microtiter plate is coated with affinity-purified anti-affinity ligand polyclonal goat antibodies.
2. Samples containing the affinity ligand are incubated in the coated plate wells.
3. Bound affinity ligand is detected by biotinylated affinity-purified anti-affinity ligand polyclonal goat antibodies.
4. Streptavidin horseradish peroxidase conjugate is added to bind to the biotinylated antibody in the sandwich complex.
5. Substrate reactive with horseradish peroxidase (tetramethylbenzidine-hydrogen peroxide) is added.
6. 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.

- 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: Use of lower-purity Bovine Serum Albumin or other blocking proteins might result in higher background levels.
- 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 (dilute immediately before use 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

Methods

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, then incubate overnight at 4°C (39°F).

Prepare standards

1. Prepare a 6.4 $\mu\text{g}/\text{mL}$ stock Standard Solution: Add 10 μL of Standard Solution (blue cap) to 770 μL of Dilution Buffer A.
2. Using the stock Standard Solution from step 1, prepare a standard dilution series according to the following table.

Tube	Conc. (ng/mL)	Standard	Dilution Buffer A
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 assay samples

Dilute 75 μL of sample with 75 μL of 2X Dilution Buffer A.

ELISA assay procedure

1. Block the plate:
 - a. Wash the coated plate 5 times with PBST.
 - b. Add 100 μL /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 μL of each concentration of the standard dilution series (0 to 64.0 ng/mL) or sample to appropriate wells.
 - b. Incubate the plate at room temperature for 1 hour on a microtiter plate shaker.
 - c. Wash the plate 5 times with PBST.
3. Add Biotinylated Reagent:
 - a. Make a 1:100 dilution of the Biotinylated Reagent with Dilution Buffer A.
 - b. Add 100 μL of diluted Biotinylated Reagent to each well and incubate the plate at room temperature for 1 hour.

c. Wash the plate 5 times with PBST.

4. Add diluted Streptavidin-Horseradish Peroxidase:
 - a. Dilute the Streptavidin-Horseradish Peroxidase in Dilution Buffer A according to the manufacturer's guidelines.
 - b. Add 100 μL of diluted 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. Add 100 μL of 1:1 mixed TMB/H₂O₂ substrate per well.
 - b. Incubate the plate for approximately 15 minutes on a microtiter plate shaker.
 - c. When the background signal starts to develop, add 50 μL of 1 M H₂SO₄ to stop the coloring reaction and achieve a maximal signal-to-noise ratio.
 - d. Measure the OD of the microtiter plate at 450 nm with a microtiter plate reader.

Calculate results

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, as this method may lead to significant inaccuracies.

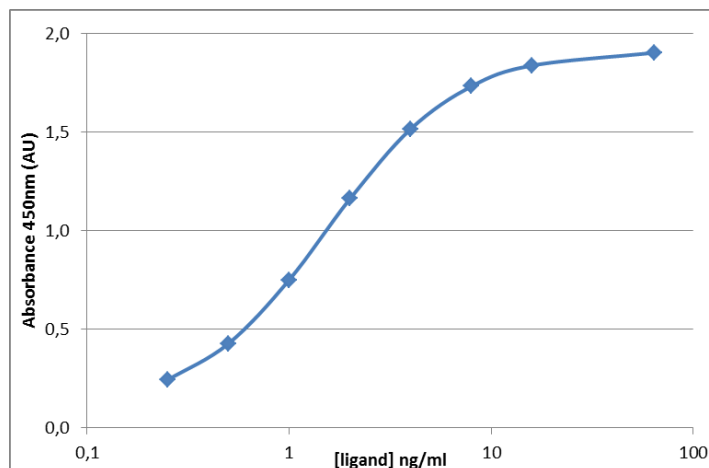


Figure 1 Example calibration curve: TSH ligand leakage assay. Results obtained using 1:2,000 diluted Streptavidin/HRP (Dako, P0379) and TMB Substrate Reagent Set (BD Biosciences, 55214).

Validate the assay

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

- Intra- and inter-assay precision experiments to establish reproducibility
- Recovery experiments using test samples with known amounts of the 500 µg/mL Standard Solution, which is included in the kit

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Manufacturer: Life Technologies Corporation | J.H. Oortweg 21 | 2333 CH Leiden | The Netherlands

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Revision	Date	Description
A.0	27 February 2018	New document.

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