CaptureSelect™ tPA Ligand Leakage ELISA

Catalog Numbers 810343001 and 810343010

Pub. No. MAN0017207 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™ tPA Ligand Leakage ELISA is designed for the detection of ≥ 1 ng/mL tPA affinity ligand that may be present in product purified with CaptureSelect™ tPA affinity media, which contains the tPA affinity ligand as the capturing agent. The tPA 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. 810343001 (1 assay)	Cat. No. 810343010 (10 assays)	Storage
Coating Reagent (green cap), Goat IgG anti-tPA affinity ligand	100 µL	1,000 µL	–20°C (–4°F)
Standard Solution (blue cap), tPA affinity ligand	100 µL	1,000 µL	
Biotinylated Reagent (yellow cap), Biotinylated Goat IgG anti-tPA 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
- (*Optional*) Dilution Buffer B: 0.05% Tween™ 20 Solution in PBS pH 7.4, 400 µM of DTT, and half the concentraion of target protein in the samples
- (*Optional*) Dilution Buffer C: 0.05% Tween™ 20 Solution in PBS pH 7.4 and 400 µM of DTT
- 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 (H_2O_2) substrate (prepare 1:1 solution immediately before use)
- 1 M H_2SO_4

- 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 the standards

1. Prepare a 6.4 µg/mL Standard Solution. Add the following to a microcentrifuge tube:

   Table 1 Standard Solution

Component	Volume
Standard Solution (blue cap)	10 µL
Dilution Buffer A	770 µL
Total	780 µL

2. Using the stock Standard Solution from step 1 and Dilution Buffer A, prepare a standard dilution series according to the following table.

   Table 2 Standard dilution series (Dilution Buffer A)

Dilution	Volume		Final concentration (ng/mL)
	Standard	Dilution Buffer A	
1	10 µL of stock Standard Solution	990 µL	64.0
2	250 µL of 64.0 ng/mL	750 µL	16.0
3	500 µL of 16.0 ng/mL	500 µL	8.0
4	500 µL of 8.0 ng/mL	500 µL	4.0
5	500 µL of 4.0 ng/mL	500 µL	2.0
6	500 µL of 2.0 ng/mL	500 µL	1.0
7	500 µL of 1.0 ng/mL	500 µL	0.5
8	500 µL of 0.5 ng/mL	500 µL	0.25
9	0	500 µL	0

Prepare the samples

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

Table 3 Sample dilution

Component	Volume
Sample	75 µL
2X Dilution Buffer A	75 µL
Total	150 µL

(*Optional*) 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 4.

   Table 4 Stock Standard Solution

Component	Volume
Standard Solution (blue cap)	10 µL
Dilution Buffer	770 µL
Total	780 µL

2. Using the stock Standard Solution from step 1, prepare a 2X concentrated standard dilution series according to Table 5.

   Table 5 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 B: Combine 0.05% Tween™ 20 in PBS (pH 7.4), 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 C: Combine 0.05% Tween™ 20 in PBS (pH 7.4) 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 20,000 x g for 5 minutes.

7. Transfer the supernatants to a new tube.

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

ELISA assay procedure

1. Block the plate:
   a. Wash the coated plate 5 times with PBST.
   b. Add 200 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 on a microtiter plate shaker.
   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/H_2O_2 substrate per well.
   b. Incubate the plate for approximately 20 minutes on a microtiter plate shaker.
   c. When the background signal starts to develop, add 50 µL of 1 M H_2SO_4 to stop the coloring reaction and achieve a maximal signal-to-noise ratio.
   d. 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, as this method may lead to significant inaccuracies.

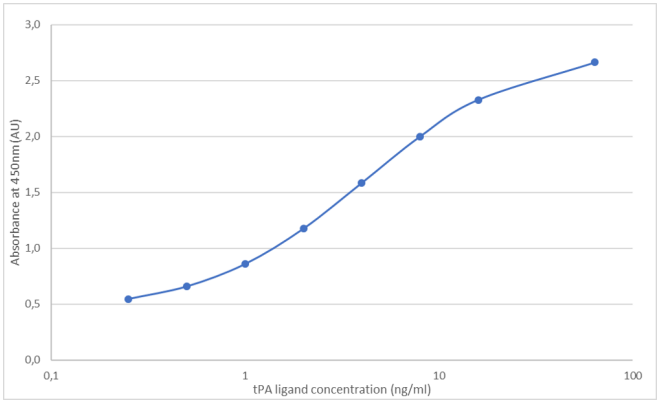

Figure 1 Example calibration curve: tPA ligand leakage assay. Results obtained using 1:2,000 diluted Streptavidin/HRP (Dako, P0379) and 1-Step™ Ultra TMB-ELISA Substrate Solution (Cat. No. 34028).

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

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

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The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0017207

Revision	Date	Description
B.0	8 December 2021	Optional sample treatment was added ("(Optional) Prepare the standards and samples: DTT treatment" on page 2). Updates were made to: • "Required materials not supplied" on page 1 • "Calculate results" on page 3
A.0	19 July 2017	New document.