CaptureSelect™ LambdaFabSelect Leakage ELISA

Catalog Numbers 810308001 and 810308010

Pub. No. MAN0015997 **Rev.** A.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™ LambdaFabSelect Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of 1 ng/mL LambdaFabSelect affinity ligand that may be present in product purified with CaptureSelect™ LambdaFabSelect affinity media, which contains the LambdaFabSelect affinity ligand as the capturing agent. The assay is designed to minimize interference and to provide accurate quantitation in the presence of polyclonal human IgG and polyclonal human Fabs. The LambdaFabSelect 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	Description	Storage
Coating Reagent (green cap)	Rabbit IgG anti-LambdaFabSelect affinity ligand, 100 µL	–20°C (–4°F)
Standard Solution (blue cap)	LambdaFabSelect affinity ligand, 100 µL	
Biotinylated Reagent (yellow cap)	Biotinylated Rabbit IgG anti-LambdaFabSelect affinity ligand, 100 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 rabbit 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 rabbit 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**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

- 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.
- 2X PBST: 0.1 (v/v)% Tween™ 20 Solution in PBS pH 7.4
- Blocking solution: 4 (w/v)% BSA in PBS pH 7.4
- Dilution Buffer A: 0.05 (v/v)% Tween™ 20 Solution in PBS pH 7.4 plus Human Fab or IgG at a concentration that is half of the concentration of target in samples
- 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 diluted Coating Reagent to each well in a microtiter plate and incubate overnight at 4°C (39°F).

Prepare standards

1. Prepare a 6.4 µg/mL stock Standard Solution: Add 10 µL Standard Solution (blue cap) to 770 µL PBST.
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 stock Standard Solution	990 µL
2	16.0	250 µL 64.0 ng/mL	750 µL
3	8.0	500 µL 16.0 ng/mL	500 µL
4	4.0	500 µL 8.0 ng/mL	500 µL
5	2.0	500 µL 4.0 ng/mL	500 µL
6	1.0	500 µL 2.0 ng/mL	500 µL
7	0.5	500 µL 1.0 ng/mL	500 µL
8	0.25	500 µL 0.5 ng/mL	500 µL
9	0	0	500 µL

Prepare assay samples

Dilute 75 µL of sample with 75 µL of 2X PBST.

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 PBST.
   b. Add 100 µL diluted Biotinylated Reagent to each well, then incubate the plate 1 hour at room temperature.
   c. Wash the plate 5 times with PBST.
4. Add diluted Streptavidin-Horseradish Peroxidase:
   a. Dilute in PBST according to the manufacturer guidelines.
   b. Add 100 µL diluted Streptavidin-Horseradish Peroxidase to each well containing sample or standard.
   c. Incubate the plate at room temperature for 1 hour 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 the TMB substrate solution according to the manufacturer guidelines.
   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 H_2SO_4 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.

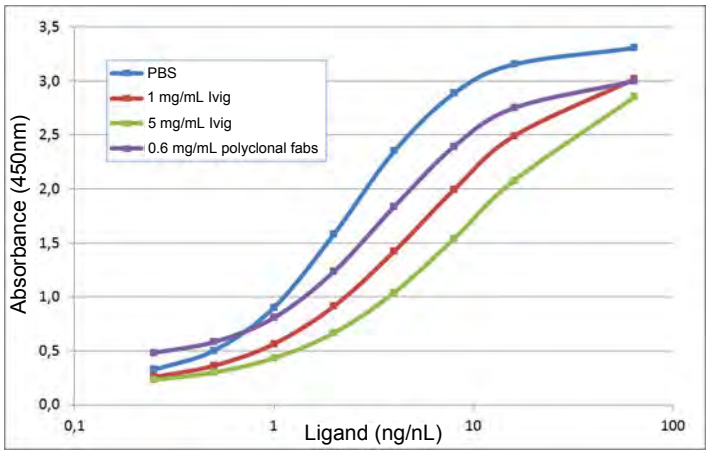

Figure 1 Example calibration curve: LambdaFabSelect leakage assay. (1) No target protein. (2) 1 mg/mL Human Ivig (3) 5 mg/mL Human Ivig (4) 0.6 mg/mL polyclonal Fabs. Results obtained using 1:2000 diluted Streptavidin/HRP (Dako, P0379) and TMB Substrate Reagent Set (BD Biosciences, 555214).

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

Ordering information

CaptureSelect™ LambdaFabSelect Leakage ELISA	Cat. No.
1 assay	810308001
10 assays	810308010

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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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.

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

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

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Revision	Date	Description
A.0	03 October 2016	New document. Replaces Pub. No. 4486472.