life technologies™

CaptureSelect™ Ig Select Leakage ELISA

INSTRUCTIONS

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Introduction

The CaptureSelect™ Ig Select Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of less than 1 ng/mL of human IgG affinity ligand that may be present in product purified with GE-Healthcare's Ig Select affinity media, which contains the human IgG affinity ligand as capturing agent. The assay is designed to minimize interference and to provide accurate quantitation in the presence of human IgG and other proteins. The Ig Select 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.

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- CaptureSelect™ Caustic stable human IgG ligand, 100 µL	-20°C (-4°F)
Standard solution (blue label)	CaptureSelect™ Caustic stable human IgG ligand, 100 µL	
Biotinylated reagent (yellow label)	Biotinylated Goat IgG anti-CaptureSelect® Caustic stable human IgG ligand, 100 µL	

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 for assays *without* target protein:
  - Dilution Buffer A: 2 (w/v)% BSA + 0.05 (v/v)% Tween® 20 Solution in PBS pH 7.4
  - 2X Dilution Buffer A: 4 (w/v)% BSA + 0.1 (v/v)% Tween® 20 Solution in PBS pH 7.4
- Dilution Buffer B for assays *with* target protein:

  PBS pH 7.4 plus Human IgG at a concentration that is half of the concentration of target in samples
- Blocking solution: 4 (w/v)% BSA in PBS pH 7.4
- Human IgG (for protocol for samples containing IgG)
- Streptavidin-Horseradish Peroxidase diluted immediately before using 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

Procedure 1: Samples without target protein

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). This step captures fragments of the leached ligand.

Prepare standards

1. Prepare a 6.4 µg/mL stock Standard solution: Add 10 µL Standard solution to 770 µL 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 µL diluted 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

1. Neutralize with 10 (v/v)% 1 M Tris pH 8.0 if samples are eluted in any of the following buffers:
   - PBS pH 2.0
   - 0.1 M Glycine pH 2.5
   - 0.1 M Acetic acid pH 2.9
   - 0.1 M Phosphoric acid pH 1.5
2. Dilute 75 µL 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 250 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 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 µL 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 (colorometric reagent that binds to the biotinylated reagents):
   a. Dilute in Dilution Buffer A according to the manufacturer's guidelines.
   b. Add 100 µL 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:H_2O_2 substrate.
   b. Add 100 µL to each well containing sample or standard.
   c. Incubate the plate for approximately 5 minutes on a microtiter plate shaker.
   d. When the background signal starts to develop, add 50 µL 1 M H_2SO_4 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.

Procedure 2: Samples with target protein

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 to 770 µL Dilution Buffer B.
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 B
1	64.0	10 µL diluted 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

1. Dilute 75 µL sample with 75 µL PBS pH 7.4.
2. Incubate the samples and standard dilution series for 15 minutes at 95°C (203°F).
3. Centrifuge the heat-treated samples and standard dilution series for 5 minutes at 20,000 *x g*.
4. Transfer the supernatants to a clean tube.

ELISA assay procedure

1. Block the plate:
   a. Wash the coated plate 5 times with PBST.
   b. Add 250 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 1 hour at room temperature on a microtiter plate shaker.
   c. Wash the plate 5 times with PBST.
3. Add Biotinylated reagents:
   a. Make a 1:100 dilution of the Biotinylated reagents with Dilution Buffer A.
   b. Add 100 µL diluted Biotinylated reagents to each well and 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 Dilution Buffer A according to the manufacturer's guidelines.
   b. Add 100 µL 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. Add 100 µL 1:1 mixed TMB/H_2O_2 substrate per well.
   b. Incubate the plate for approximately 5 minutes on a microtiter plate shaker.
   c. When the background signal starts to develop, add 50 µL 1M 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, which may lead to significant inaccuracies.

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 µg/mL Standard solution, which is included in the kit.

Ordering information

CaptureSelect™ Ig Select Leakage ELISA	Part Number
1 assay	810083501
10 assays	810083510

For more information

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

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.

Limited Product Warranty

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