CaptureSelect™ IgG-CH1 Ligand Leakage ELISA

INSTRUCTIONS

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Introduction

The CaptureSelect™ IgG-CH1 Ligand Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of less than 1 ng/mL of human IgG-CH1 affinity ligand that may be present in product purified with IgG-CH1 affinity media, which contains the IgG-CH1 affinity ligand as capturing agent. The assay is designed to minimize interference and to provide accurate quantitation in the presence of human IgG and Fab fragments. The IgG-CH1 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.

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 Ig anti-IgG-CH1 affinity ligand, 100 µL	-20°C (-4°F)
Standard solution (blue label)	CaptureSelect™ CH1 affinity ligand, 100 µL	
Biotinylated reagent (yellow label)	Biotinylated Goat IG anti-IgG-CH1 affinity 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: 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
- Dilution Buffer B for assays *with* target protein:

  0.1 (v/v)% Tween® 20 Solution in PBS pH 7.4 plus 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 dilution series: 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

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

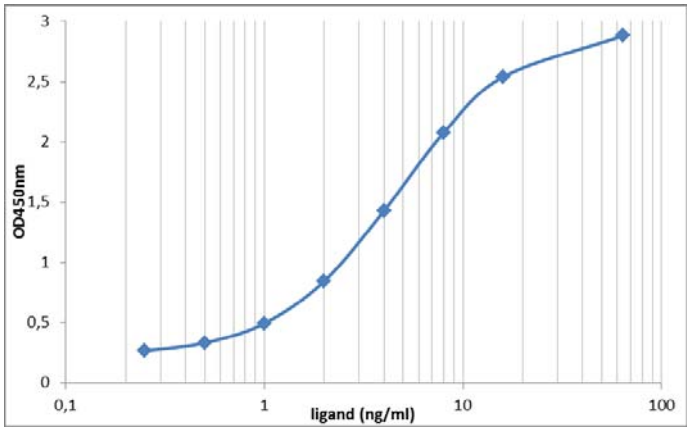
ELISA assay procedure

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 15 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.

Figure 1 Example calibration curve CaptureSelect™ IgG-CH1 Ligand Leakage ELISA without target protein.



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 2X Dilution Buffer A.
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.

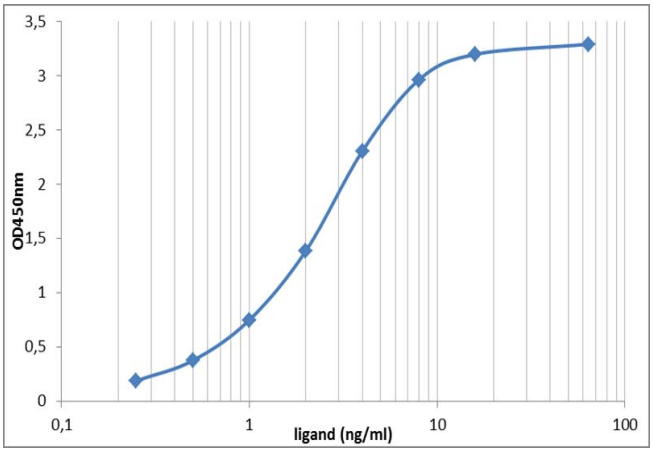
ELISA assay procedure

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 50 minutes on a microtiter plate shaker.
   c. 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.
   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.

Figure 2 Example calibration curve CaptureSelect™ IgG-CH1 Ligand Leakage ELISA in the presence of 1 mg/mL human IgG.



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™ IgG-CH1 Ligand Leakage ELISA	Part Number
1 assay	810320001
10 assays	810320010

For more information

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

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.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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.

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