

CaptureSelect™ Alpha-1 Antitrypsin Select Leakage ELISA

INSTRUCTIONS

Publication Number 4486420 Rev. A

- Introduction..... 1
- Principle of the assay 1
- Kit contents 1
- Required materials and equipment (not provided)..... 1
- Procedure 1: Samples without target protein..... 2
- Procedure 2: Samples with target protein..... 2
- Validate the assay 3
- Ordering information..... 4
- For more information..... 4
- Safety information 4
- Limited Product Warranty 4

Introduction

The CaptureSelect™ Alpha-1 Antitrypsin Select Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of less than 1 ng/mL of AAT affinity ligand that may be present in product purified with GE Healthcare's Alpha-1 Antitrypsin Select affinity media, which contains the AAT affinity ligand as capturing agent. The assay is designed to minimize interference and to provide accurate quantitation in the presence of Alpha-1 Antitrypsin and other proteins. The Alpha-1 Antitrypsin 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-AAT Select affinity ligand, 500 µL	-20°C (-4°F)
Standard solution (blue label)	CaptureSelect™ human AAT affinity ligand, 100 µL	
Biotinylated reagent (yellow label)	Biotinylated Goat IgG anti-AAT Select 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 >98% 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: 1:1 mixture of 20 mM Tris, 2.0 M MgCl₂ + PBS pH 7.4 plus Human AAT at a concentration that is half of the concentration of target in samples
- AAT Select elution buffer: 20 mM Tris, 2.0 M MgCl₂ pH 7.0
- Blocking solution: 4 (w/v)% BSA in PBS pH 7.4
- Human AAT (for protocol for samples containing human AAT; Sigma-Aldrich A6150)
- Streptavidin-Horseradish Peroxidase diluted immediately before using 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

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 $\mu\text{g}/\text{mL}$ stock Standard dilution series: Add 5 μL Standard solution to 775 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

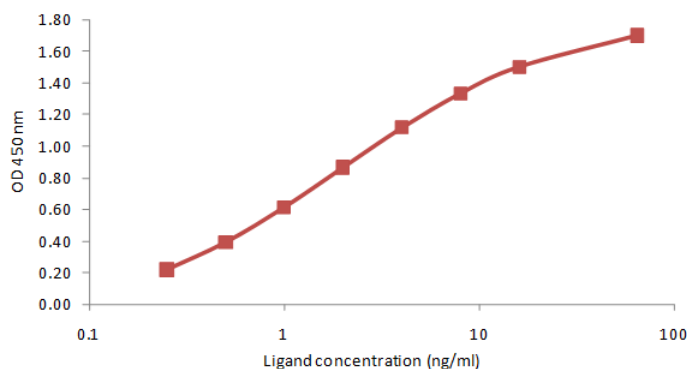
1. Block the plate:
 - a. Wash the coated plate 5 times with PBST.
 - b. Add 250 μL /well of Blocking solution to the coated plate. Leave at room temperature for 30 minutes on a microtiter plate shaker.
 - c. Wash the plate 1 time with PBST.
2. Add samples and standards:
 - a. Add 100 μL of each concentration 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 (colorimetric 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:H₂O₂ 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 H₂SO₄ 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 Alpha-1 Antitrypsin Select leakage assay for samples without human AAT and magnesium chloride.



Procedure 2: Samples with target protein

Coat the plate

1. Make a 1:20 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 5 µL Standard solution to 775 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

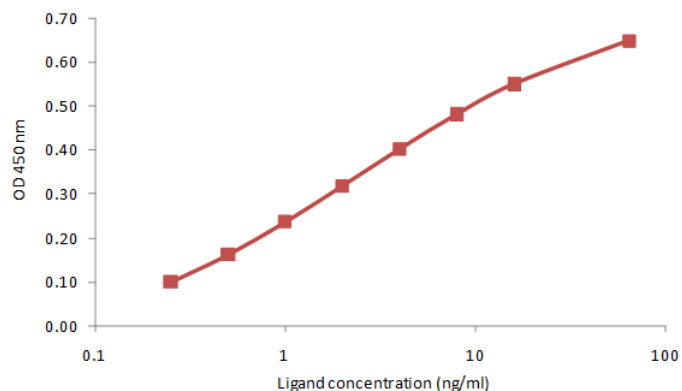
1. Block the plate:
 - a. Wash the coated plate 5 times with PBST.
 - b. Add 250 µL/well of Blocking solution to the coated plate. Leave at room temperature for 30 minutes on a microtiter plate shaker.
 - c. Wash the plate 1 time with PBS pH 7.4 + 0.05 (v/v)% Tween[®] 20 Solution.
2. Add samples and standards:
 - a. Add 100 µL supernatant of the centrifuged samples or standard dilution series per well.
 - b. Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
 - c. Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween[®] 20 Solution.
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/H₂O₂ 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 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 the 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 Alpha-1 Antitrypsin Select leakage assay in the presence of 5 mg/mL human AAT and magnesium chloride.



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

Ordering information

CaptureSelect™ Alpha-1 Antitrypsin Select Leakage ELISA	Part Number
1 assay	810287001
10 assays	810287010

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

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.

For Research Use Only. Not for use in diagnostic procedures.

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

DISCLAIMER: LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners. Tween is a registered trademark of Uniqema Americas, LLC. Milli-Q is a registered trademark of Merck KGaA.

© Copyright 2013, Life Technologies Corporation. All rights reserved.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com
lifetechnologies.com

June 2013

