


# CaptureSelect™ AAVX Ligand Leakage ELISA

Catalog Numbers 810352201 and 810352210

Pub. No. MAN0017417 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](http://thermofisher.com/support).

## Product description

The CaptureSelect™ AAVX Ligand Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of 1 ng/mL AAVX affinity ligand that may be present in product purified with POROS™ CaptureSelect™ AAVX affinity media, which contains the AAVX affinity ligand as the capturing agent. The AAVX 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. 810352201 (1 assay)	Cat. No. 810352210 (10 assays)	Storage
Coating Reagent (green cap), Goat IgG anti-AAVX affinity ligand	100 µL	1,000 µL	-20°C (-4°F)
Standard Solution (blue cap), AAVX affinity ligand	100 µL	1,000 µL	
Biotinylated Reagent (yellow cap), Biotinylated Goat IgG anti-AAVX 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](http://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
- 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<sub>2</sub>O<sub>2</sub>) substrate (prepare 1:1 solution immediately before use)
- 1 M H<sub>2</sub>SO<sub>4</sub>
- 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  $\mu\text{L}$  of diluted Coating Reagent to each well in a microtiter plate, then incubate overnight at 4°C (39°F).

### Prepare standards

1. Prepare a 6.4  $\mu\text{g}/\text{mL}$  stock Standard Solution: Add 10  $\mu\text{L}$  of Standard Solution (blue cap) to 770  $\mu\text{L}$  of Dilution Buffer A.
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 $\mu\text{L}$ of stock Standard Solution	990 $\mu\text{L}$
2	16.0	250 $\mu\text{L}$ of 64.0 ng/mL	750 $\mu\text{L}$
3	8.0	500 $\mu\text{L}$ of 16.0 ng/mL	500 $\mu\text{L}$
4	4.0	500 $\mu\text{L}$ of 8.0 ng/mL	500 $\mu\text{L}$
5	2.0	500 $\mu\text{L}$ of 4.0 ng/mL	500 $\mu\text{L}$
6	1.0	500 $\mu\text{L}$ of 2.0 ng/mL	500 $\mu\text{L}$
7	0.5	500 $\mu\text{L}$ of 1.0 ng/mL	500 $\mu\text{L}$
8	0.25	500 $\mu\text{L}$ of 0.5 ng/mL	500 $\mu\text{L}$
9	0	0	500 $\mu\text{L}$

### Prepare assay samples

Dilute 75  $\mu\text{L}$  of sample with 75  $\mu\text{L}$  of 2X Dilution Buffer A.

### ELISA assay procedure

1. Block the plate:
  - a. Wash the coated plate 5 times with PBST.
  - b. Add 100  $\mu\text{L}$ /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  $\mu\text{L}$  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  $\mu\text{L}$  of diluted Biotinylated Reagent to each well and incubate the plate at room temperature for 1 hour.

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  $\mu\text{L}$  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  $\mu\text{L}$  of 1:1 mixed TMB/H<sub>2</sub>O<sub>2</sub> substrate per well.
  - b. Incubate the plate for approximately 15 minutes on a microtiter plate shaker.
  - c. When the background signal starts to develop, add 50  $\mu\text{L}$  of 1 M H<sub>2</sub>SO<sub>4</sub> 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.

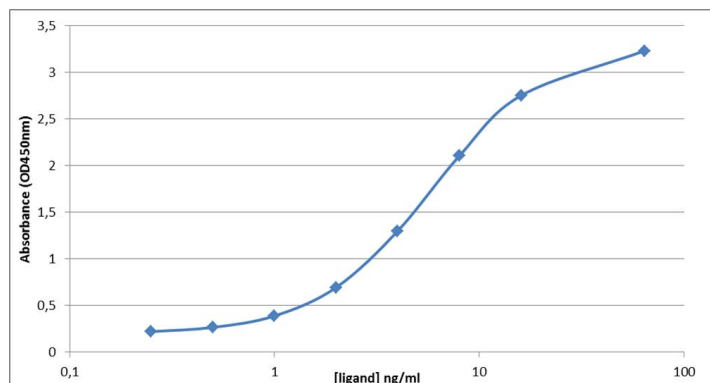


Figure 1 Example calibration curve: AAVX ligand leakage assay. Results obtained using 1:2,000 diluted Streptavidin/HRP (Dako, P0379) and TMB Substrate Reagent Set (BD Biosciences, 55214).

## 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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Revision	Date	Description
A.0	30 October 2017	New document.

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