# CaptureSelect™ AVB Sepharose HP Leakage ELISA

Catalog Numbers 810280801 and 810280810

Pub. No. MAN0018252 Rev. C

**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™ AVB Sepharose HP Leakage ELISA is designed to detect possible leached ligand of AAV capsids purified using AVB Sepharose HP resin (Cytiva). The ELISA provides typical sensitivity and accurate quantitation of possible leached ligand ≥1 ng/mL.

## Contents and storage

| Contents | Cat. No. 810280801 (1 assay) | Cat. No. 810280810 (10 assays) | Storage |
|---|---|---|---|
| Coating Reagent (green cap), Goat IgG anti-AVB Sepharose HP affinity ligand | 100 µL | 1,000 µL | –20°C (–4°F) |
| Standard Solution (blue cap), AVB Sepharose HP affinity ligand | 100 µL | 1,000 µL | |
| Biotinylated Reagent (yellow cap), Biotinylated Goat IgG anti-AVB Sepharose HP affinity ligand | 100 µL | 1,000 µL | |

## Principle of the assay

AVB ligand that is immobilized on chromatography media can leach off the resin and co-elute with the target protein. The CaptureSelect™ AVB Sepharose HP Leakage ELISA assay enables detection of the affinity ligand.

The assay is a sandwich-type ELISA that uses a horseradish peroxidase detection system and involves the following steps:

1. The AVB ligand that is present in the samples and prepared standard dilution series is captured by affinity-purified anti-affinity ligand polyclonal goat antibodies coated on the plate.
2. The bound affinity ligand is detected by adding biotinylated affinity-purified anti-affinity ligand polyclonal goat antibodies.
3. Streptavidin-Horseradish Peroxidase conjugate is added to bind to the biotinylated antibody in the sandwich complex.
4. Substrate reactive with horseradish peroxidase [tetramethylbenzidine (TMB)] is added.
5. 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.

Catalog numbers that appear as links open the web pages for those products.

### Critical materials

Critical materials have a significant impact on the assay performance. To reduce assay development and optimization efforts, recommendations for the critical materials are listed in Table 1.

**Table 1 Critical materials**

| Item | Source |
|---|---|
| **Blocking reagent** | |
| StartingBlock™ (PBS) Blocking Buffer | 37578 |
| Blocker™ Casein in PBS | 37528 |
| Bovine Serum Albumin (BSA), Fraction V 99% pure | Sigma-Aldrich™ A3059 |
| StabilCoat™ Immunoassay Stabilizer | SURMODICS SC01 |
| **Microtiter plate** | |
| Nunc™ MaxiSorp™ flat-bottom | 442404 |
| **Streptavidin-Horseradish Peroxidase conjugate** | |
| HRP-Conjugated Streptavidin | N100 |
| Streptavidin/HRP (Conjugate) | Agilent Dako p3097 |

| Item | Source |
|---|---|
| **Tetramethylbenzidine (TMB) substrate** | |
| 1-Step™ Ultra TMB-ELISA Substrate Solution | 34028 |
| TMB Substrate Solution | N301 |
| ELISA TMB Stabilized Chromogen | SB02 |
| BD OptEIA™ TMB Substrate Reagent Set | BD Biosciences 555214 |

### Non-critical materials

Non-critical materials have limited to no impact on the assay performance. You can perform the assay with the non-critical materials you may already have.

- PBS: Phosphate buffered saline pH 7.4
- PBST: Phosphate buffered saline (PBS) pH 7.4 + 0.05 (v/v)% Tween™ 20 Solution
- Demineralized water
- 0.2M–1M $H_2SO_4$
- Pipettes, 10–100 µL
- Multichannel pipettes, 100 µL
- Microtiter plate reader spectrophotometer with wavelength capability at 450 nm
- Microtiter plate shaker
- Microcentrifuge tubes

## Before you begin

**IMPORTANT!** The assay should be performed by technically qualified individuals.

- Read through the entire procedure.
- Bring all reagents to room temperature before use.
- Prepare other materials as needed (see “Required materials not supplied” on page 1).
- Prepare the standard dilution series according to the assay protocol.
- Set the plate reader to 450-nm wavelength.
- Locate the assay certificate (see “Locate the assay certificate” on page 2).

### Locate the assay certificate

Each ELISA assay lot is performance tested, and the results are captured in an assay certificate. The assay certificate provides the reagent concentrations, internal control recovery, an example calibration curve, and the recommended TMB incubation time.

1. Go to **www.thermofisher.com**.
2. In the search bar, enter ***certificate*** `<lot number>`, where `<lot number>` is the lot number for your ELISA assay (for example, 220822-101).

## Procedural guidelines

- To achieve good assay reproducibility and sensitivity, you must wash the plate extensively after each step.
- Sample components (such as pH, salts, impurities, or the target protein itself) can affect the assay performance. The effects should be studied in order to yield acceptable background signals, sensitivity, and recovery. To correct for these components, add them to the standard dilution series in similar concentrations. Ensure that these additions do not contain leached ligand, which will affect the assay performance. If these additions are not sufficient, investigate the other sample preparation methods described in “Optional sample treatment protocols” on page 4.
- During optimization and validation of the leakage ELISA, the TMB incubation time is an important parameter to investigate. As a guideline for the TMB incubation time, you can use the time specified on the lot-specific assay certificate; however, small deviations may be required, based on your process.
- After the TMB incubation is stopped using the stop solution, measure the plate within 5 minutes. After 5 minutes, black precipitation can occur in the wells and affect the assay performance.
- To prevent systematic inaccuracies, maintain a constant timing sequence from well-to-well, especially when adding the TMB and stop solution.

### Manually wash the plates *(recommended procedure)*

1. Empty the contents of the wells into a waste bin or sink.
2. Gently but firmly tap the plate upside down on a paper towel or other absorbent material to remove most of the residual liquid.

   **Note:** Tapping too hard may dislodge the antibody bound material from the plate and cause elevated background signals and overall lower signals.
3. Use a squirt bottle to completely fill the wells with PBST.

   **Note:** A squirt bottle is preferable, but you can also use a pipette to fill the wells.

## Methods

**Note:** After thawing and before use, spin the tubes to ensure that all reagents are at the bottom of the tube.

### Coat the plate

1. Make a 1:100 dilution of the Coating Reagent with PBS pH 7.4.
2. Add 100 µL of diluted Coating Reagent to each well in a microtiter plate, then incubate overnight at 4°C (39°F).
3. Incubate the plate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature. Alternatively, incubate the plate overnight at 4°C (39°F), without shaking.

## Prepare standards

1. Prepare a 6.4 µg/mL stock Standard Solution: Add components to a microcentrifuge tube according to Table 2.

**Table 2 Stock Standard Solution**

| Component | Volume |
|---|---|
| Standard Solution (blue cap) | 10 µL |
| PBST | 770 µL |
| **Total** | **780 µL** |

2. Using the stock Standard Solution from step 1, prepare a standard dilution series according to Table 3.

**Table 3 Standard dilution series**

| Tube | Conc. (ng/mL) | Standard | PBST |
|---|---|---|---|
| 1 | 64.0 | 10 µL of stock Standard Solution | 990 µL |
| 2 | 16.0 | 250 µL of 64.0 ng/mL | 750 µL |
| 3 | 8.0 | 500 µL of 16.0 ng/mL | 500 µL |
| 4 | 4.0 | 500 µL of 8.0 ng/mL | 500 µL |
| 5 | 2.0 | 500 µL of 4.0 ng/mL | 500 µL |
| 6 | 1.0 | 500 µL of 2.0 ng/mL | 500 µL |
| 7 | 0.5 | 500 µL of 1.0 ng/mL | 500 µL |
| 8 | 0.25 | 500 µL of 0.5 ng/mL | 500 µL |
| 9 | 0 | 0 | 500 µL |

## Prepare the samples

Dilute the sample by adding the following to a microcentrifuge tube:

**Table 4 Sample dilution**

| Component | Volume |
|---|---|
| Sample | 75 µL |
| 2X Dilution Buffer A | 75 µL |
| **Total** | **150 µL** |

## ELISA assay procedure

**IMPORTANT!** For all wash steps, manually wash the plate according to “Manually wash the plates (recommended procedure)” on page 2.

1. Block the plate:
   a. Wash the plate 1 time with PBST.
   b. Add 200 µL/well of Blocking solution to the coated plate.
   c. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 30 minutes at room temperature.
2. Add samples and standards:
   a. Wash the plate 1 time with PBST.
   b. Add 100 µL of each concentration of the standard dilution series (0 to 64.0 ng/mL) or sample to the appropriate wells.
   c. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature.
3. Add Biotinylated Reagent:
   a. Wash the plate 3 times with PBST.
   b. Make a 1:100 dilution of the Biotinylated Reagent with PBST.
   c. Add 100 µL of the diluted Biotinylated Reagent to each well.
   d. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature.
4. Add diluted Streptavidin-Horseradish Peroxidase conjugate:
   a. Wash the plate 3 times with PBST.
   b. Dilute the Streptavidin-Horseradish Peroxidase conjugate with PBST according to the manufacturer guidelines.
   c. Add 100 µL of the diluted peroxidase to each well.
   d. Cover the plate, then incubate on a microtiter plate shaker at 200–500 rpm for 1 hour at room temperature.
5. Develop and read the plate:
   a. Wash the plate 3 times with PBST.
   b. Wash the plate 2 times with demineralized water.
   c. Add 100 µL of TMB substrate to each well.
   d. Cover the plate, then incubate on a microtiter plate shaker for the recommended incubation time at room temperature.

      **Note:** The recommended incubation time is stated on the assay certificate. See “Locate the assay certificate” on page 2.
   e. Add 50 µL of 0.2–1 M $H_2SO_4$ to each well to stop the reaction.
   f. Within 5 minutes, measure the absorbance of the microtiter plate at 450 nm with a microtiter plate reader.

### Calculate results

1. 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.

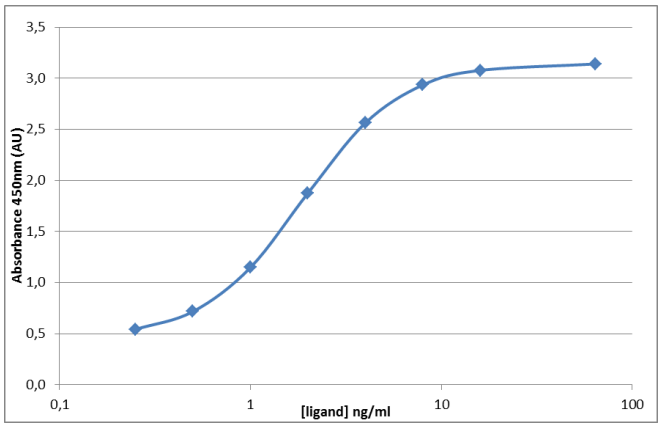


**Figure 1 Example calibration curve: AVB Sepharose HP leakage assay. Results obtained using 1:2,000 diluted Streptavidin/HRP (Dako, P0379) and TMB Substrate Reagent Set (BD Biosciences, 55214).**

2. Use the standard curve and the sample absorbance to determine the ligand concentration in your sample.

### Calculate results

1. 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, because this method may lead to significant inaccuracies.

2. Use the standard curve and the sample absorbance to determine the ligand concentration in your sample.

Information about the calibration curve obtained during the quality assessment, including the reagents and incubation time used, is provided in the assay certificate. See “Locate the assay certificate” on page 2.

## Validate the assay

Perform validation studies that include at least the following experiments to validate this assay for your application:

- Intra- and inter-assay precision experiments to establish reproducibility.
- Recovery experiments using test samples with known amounts of AVB ligand. The test samples can be prepared from the 500 µg/mL Standard Solution that is included in the kit.

  **Note:** The test samples can be aliquoted and stored at -20°C for long-term storage.

## Optional sample treatment protocols

Because every protein purified with the affinity resins and purification process can be different, matrix effects on the AVB Sepharose HP Ligand Leakage ELISA assay are difficult to predict. If correcting for the buffer matrix influences is not sufficient for obtaining good recoveries, you may need to perform one of the following sample treatment options.

**Note:** These sample treatment protocols have not been tested with the AVB Sepharose HP Ligand Leakage ELISA assay.

### Prepare the standards and samples: Heat treatment

Heat treatment is performed on the sample and standard dilution series to remove the target protein by precipitation while the ligand stays in solution, increasing assay performance and recovery.

1. Prepare a 6.4 µg/mL stock Standard Solution: Add components to a microcentrifuge tube according to Table 5.

**Table 5 Stock Standard Solution**

| Component | Volume |
|---|---|
| Standard Solution (blue cap) | 10 µL |
| PBST | 770 µL |
| Total | 780 µL |

2. Using the stock Standard Solution from step 1, prepare a 2X concentrated standard dilution series according to Table 6.

**Table 6 Standard dilution series**

| Tube | Conc. (ng/mL) | Standard | PBST |
|---|---|---|---|
| 1 | 128.0 | 20 µL of stock Standard Solution | 980 µL |
| 2 | 32.0 | 250 µL of 64.0 ng/mL | 750 µL |
| 3 | 16.0 | 500 µL of 16.0 ng/mL | 500 µL |
| 4 | 8.0 | 500 µL of 8.0 ng/mL | 500 µL |
| 5 | 4.0 | 500 µL of 4.0 ng/mL | 500 µL |
| 6 | 2.0 | 500 µL of 2.0 ng/mL | 500 µL |
| 7 | 1.0 | 500 µL of 1.0 ng/mL | 500 µL |
| 8 | 0.5 | 500 µL of 0.5 ng/mL | 500 µL |
| 9 | 0 | 0 | 500 µL |

3. Dilute the standards from the dilution series 1:1 with Dilution Buffer A.

   To prepare Dilution Buffer A: Combine PBST and target protein. Use target protein with a concentration comparable to the samples.

4. Dilute the samples 1:1 with PBST.

5. Incubate the standards and samples for 15 minutes at 95°C, then allow to cool to room temperature.

6. Centrifuge the standards and samples at 20,000 × *g* for 5 minutes.

7. Transfer the supernatant to a clean tube.

8. Proceed to “ELISA assay procedure” on page 3.

### Prepare the standards and samples: DTT treatment

DTT is added to the sample and standard dilution series to reduce the ligand-target protein complex, increasing assay performance and recovery.

1. Prepare a 6.4 µg/mL stock Standard Solution: Add components to a microcentrifuge tube according to Table 7.

**Table 7 Stock Standard Solution**

| Component | Volume |
|---|---|
| Standard Solution (blue cap) | 10 µL |
| PBST | 770 µL |
| Total | 780 µL |

2. Using the stock Standard Solution from step 1, prepare a 2X concentrated standard dilution series according to Table 8.

**Table 8 Standard dilution series**

| Tube | Conc. (ng/mL) | Standard | PBST |
|---|---|---|---|
| 1 | 128.0 | 20 µL of stock Standard Solution | 980 µL |
| 2 | 32.0 | 250 µL of 64.0 ng/mL | 750 µL |
| 3 | 16.0 | 500 µL of 16.0 ng/mL | 500 µL |
| 4 | 8.0 | 500 µL of 8.0 ng/mL | 500 µL |
| 5 | 4.0 | 500 µL of 4.0 ng/mL | 500 µL |
| 6 | 2.0 | 500 µL of 2.0 ng/mL | 500 µL |
| 7 | 1.0 | 500 µL of 1.0 ng/mL | 500 µL |
| 8 | 0.5 | 500 µL of 0.5 ng/mL | 500 µL |
| 9 | 0 | 0 | 500 µL |

3. Dilute the standards from the dilution series 1:1 with Dilution Buffer A.

   To prepare Dilution Buffer A: Combine PBST, target protein, and 400 µM of DTT. Use target protein with a concentration comparable to the samples.

4. Dilute the samples 1:1 with Dilution Buffer B.

   To prepare Dilution Buffer B: Combine PBST and 400 µM of DTT.

5. Incubate the standards and samples for 1 hour at 60°C, then allow to cool to room temperature.

6. To ensure that no liquid is in the tube caps, centrifuge the standards and samples at 5,000 rpm for 30 seconds.

7. Proceed to “ELISA assay procedure” on page 3.

## Limited product warranty

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    **Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

**Revision history:** Pub. No. MAN0018252 C

| Revision | Date | Description |
|---|---|---|
| C | 23 July 2024 | Update formatting and content to meet current Leakage ELISA guidelines. |
| B.0 | 12 April 2019 | Update to lot number and the volume of Blocking solution used in the ELISA assay procedure. |
| A.0 | 14 December 2018 | New document. |