

CaptureSelect™ AAV9 Ligand Leakage ELISA

Catalog Number 810333001 and 810333010

Pub. No. MAN0010764 Rev. A

Item	Description	Storage conditions
Coating reagent (green label)	Goat IgG anti-AAV9 affinity ligand, 100 µL	-20°C (-4°F)
Standard solution (blue label)	AAV9 affinity ligand, 100 µL	
Biotinylated reagent (yellow label)	Biotinylated Goat IgG anti-AAV9 affinity ligand, 100 µL	

 **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 www.lifetechnologies.com/support.

Product description

The CaptureSelect™ AAV9 Ligand Leakage ELISA (Enzyme Linked Immuno-Sorbent Assay) is designed for the detection of 1 ng/mL AAV9 affinity ligand that may be present in product purified with CaptureSelect™ AAV9 affinity media, which contains the AAV9 affinity ligand as capturing agent. The assay is designed to minimize interference and to provide accurate quantitation in the presence of Adeno-associated virus particles and other proteins. The AAV9 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™ 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:

- A microtiter plate is coated with affinity-purified anti-affinity ligand polyclonal goat antibodies.
- Samples containing the affinity ligand are incubated in the coated plate wells.
- Bound affinity ligand is detected by biotinylated affinity-purified anti-affinity ligand polyclonal goat antibodies.
- 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.

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: 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 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

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 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 200 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/H₂O₂ substrate per well.
 - b. Incubate the plate for approximately 20 minutes on a microtiter plate shaker.
 - c. 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.
 - 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.

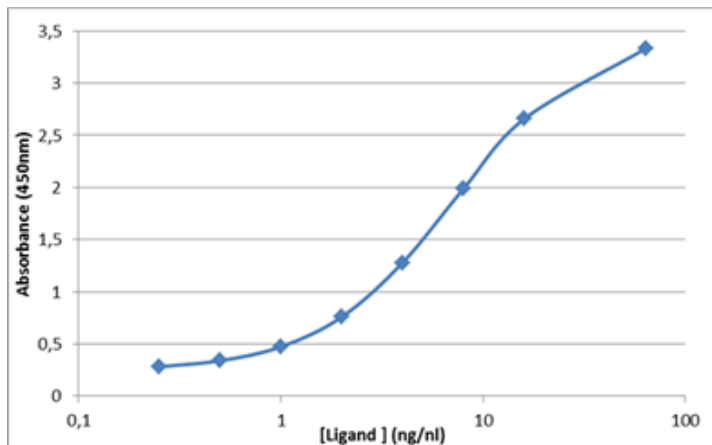


Figure 1 Example calibration curve AAV9 ligand leakage assay

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 $\mu\text{g/mL}$ Standard solution, which is included in the kit.

Ordering information

CaptureSelect™ AAV9 Ligand Leakage ELISA	Part number
1 assay	810333001
10 assays	810333010

For more information

For more information on CaptureSelect™ products and ligand leakage ELISA products, go to www.lifetechnologies.com/captureselect.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Note: For SDSs of chemicals from third-party manufacturers, contact the chemical manufacturer.

Limited product warranty

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