VIIISelect leakage ELISA

Enzyme Linked Immuno-Sorbent Assay for the measurement of CaptureSelect B domain depleted recombinant Factor VIII affinity ligand.

Intended Use

This kit is intended for use in quantifying VIIISelect affinity ligand. The kit is for research and manufacturing use only and is not intended for diagnostic use.

Introduction

VIIISelect affinity medium (GE Healthcare) is designed for the purification of B domain depleted recombinant Factor VIII. The CaptureSelect B domain depleted recombinant Factor VIII affinity ligand is based on a single chain antibody fragment that was developed by screening for unique selectivity for human Factor VIII. The ligand, a 13 kD recombinant protein, is coupled with multipoint attachment through stable amide bonds to give high chemical stability and low leakage. Even when covalently attached, small amounts of the VIIISelect affinity ligand can leach off of the chromatography support and co-elute with human Factor VIII. This ELISA provides sensitivity to detect VIIISelect affinity ligand contamination to less than 1 ng/ml. This assay is designed to minimize interference and to provide accurate quantitation in the presence of human Factor VIII. This kit 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 VIIISelect leakage assay is designed to detect VIIISelect affinity ligand in solution. The assay is a sandwich ELISA, utilizing microtiter plates coated with affinity purified goat anti-VIIISelect affinity ligand. Samples containing VIIISelect affinity ligand are incubated in microtitre plate coated wells. Unbound materials are removed by washing. Bound VIIISelect affinity ligand is detected by biotinylated goat anti-VIIISelect affinity ligand. After incubation excess conjugate is washed out and a streptavidin horseradish peroxidase conjugate is added. This will bind to any of the biotinylated antibody of the sandwich complex. After washing, a substrate reactive with horseradish peroxidase is added (tetramethylbenzidine-hydrogenperoxide). The amount of hydrolyzed substrate is read on a microtiter plate reader and will be directly proportional to the concentration of VIIISelect affinity ligand present. Accurate quantitation is achieved by comparing the signal of unknowns to VIIISelect affinity ligand standards assayed at the same time.

Warranty: VIIISelect leakage ELISA of BAC BV is supplied for research and manufacturing use only and is intended to be used by a technically qualified individual. If you are not satisfied with the performance of a BAC BV product, please contact BAC BV.

Provided Materials

* Coating reagents: 100 µl Goat IgG anti-VIIISelect affinity ligand
* Standard solution: 100 µl VIIISelect affinity ligand
* Biotinylated reagents: 100 µl Biotinylated Goat IgG anti-VIIISelect affinity ligand

Upon arrival all reagents should be stored at -20 °C for stability.

Required Materials and Equipment (not provided)

* Phosphate buffered saline (PBS) pH 7.4
* Tween 20
* Bovine Serum Albumin (BSA), Fraction V 99% pure (Sigma-Aldrich)
  Note: *Other qualities of Bovine Serum Albumin or other blocking proteins might result in higher background levels.*
* Streptavidin-Horseradish Peroxidase
* Tetramethylbenzidine (TMB) and Hydrogenperoxide (H_2O_2) substrate
* 1 M H_2SO_4
* Microtiter plate (Maxisorp, Nunc)
* Microtiter plate shaker
* Microtiter plate reader (450 nm)

Protocols and results

The protocols and data described below were generated by BAC BV to establish the performance and validity of the materials in the ELISA kit. This data is intended to supplement and not to replace user generated validation data. The data is representative of what a laboratory can expect to achieve when the described materials and protocols are used. It is recommended that a user validation study include at least the following experiments to validate this kit with their product: 1) Each user should perform intra and inter assay precision experiments to establish their reproducibility. 2) Each user should perform recovery experiments using their test samples with known amounts of the 500 µg/ml standard solution which is included in the kit.

Protocol

- Dilute the Coating reagents 100 times with PBS pH 7.4.
- Add 100 µl diluted Coating reagents to each well and incubate the microtiter plate overnight at 4 °C (39 °F).
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Block the plate for 30 minutes at room temperature with 250 µl/well 4 (w/v)% BSA in PBS pH 7.4 on a microtiter plate shaker.
- Wash the plate 1 time with PBS pH 7.4 + 0.05 (v/v)% Tween 20.

- Prepare a standard dilution serie by diluting the Standard solution. Recommended is 1000 times to 500 ng/ml in dilution buffer containing 2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4, followed by dilutions with a factor three.

1: [500] = 1 µl Standard solution + 999 µl dilution buffer
2: [167] = 300 µl [500] + 600 µl dilution buffer
3: [56] = 300 µl [167] + 600 µl dilution buffer
Up to
8: [0.23] = 300 µl [0.69] + 600 µl dilution buffer

- Dilute 75 µl sample with 75 µl 2 times concentrated dilution buffer (4 (w/v)% BSA + 0.1 (v/v)% Tween 20 in PBS pH 7.4).

- Add 100 µl standard or sample per well.
- Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Dilute the Biotinylated reagents 100 times with 2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4.
- Add 100 µl diluted Biotinylated reagents to each well and incubate the plate 1 hour at room temperature.
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Add 100 µl 1:4000 diluted Streptavidin-Horseradish peroxidase in 2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4.
- Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Wash the plate 2 times with demineralized water.
- Add 100 µl 1:1 mixed TMB/H_2O_2 substrate per well.
- Incubate the plate for approximately 10 minutes on a microtiter plate shaker.
- Stop the coloring reaction with 50 µl 1M H_2SO_4.
- Measure the OD of the microtiter plate at 450 nm with a microtiter plate reader.

Calculation of Results

The standards are used to construct a standard curve with values reported in ng/mL. This data reduction may be performed through computer methods using curve fitting routines such as 4 parameter logistic fit. Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies.

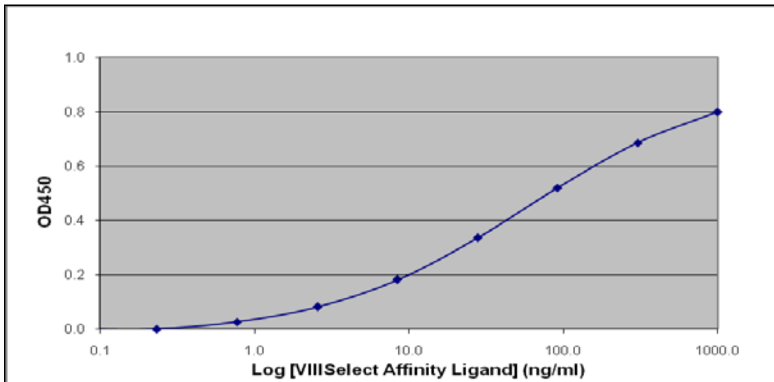

Figure 1: Calibration curve VIIISelect leakage assay.

Contact

For further information or questions about the VIIISelect leakage ELISA please send an E-mail to ligands@captureselect.com