IgSelect leakage ELISA

Enzyme Linked Immuno-Sorbent Assay for the Measurement of CaptureSelect Caustic Stable Human IgG Affinity Ligand.

Intended Use

This kit is intended for use in quantifying CaptureSelect Caustic Stable Human IgG Affinity Ligand. The kit is for research and manufacturing use only and is not intended for diagnostic use.

Introduction

IgSelect affinity medium (GE Healthcare) is designed for the purification of human IgG from different sources. The IgSelect affinity ligand is based on a single chain antibody fragment that was developed by screening for chemical stability and unique selectivity for Fc fragments of human IgG (CaptureSelect Caustic Stable Human IgG Affinity Ligand). 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 IgSelect affinity ligand can leach off of the chromatography support and co-elute with the antibody. This ELISA provides sensitivity to detect IgSelect 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 immunoglobulins. 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 IgSelect leakage assay is designed to detect IgSelect affinity ligand in solutions with and without IgG. The assay is a sandwich ELISA, utilizing microtiter plates coated with affinity purified goat anti-IgSelect affinity ligand. Samples containing IgSelect affinity ligand are incubated in microtitre plate coated wells. Unbound materials are removed by washing. Bound IgSelect affinity ligand is detected by biotinylated goat anti-IgSelect 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 IgSelect affinity ligand present. Accurate quantitation is achieved by comparing the signal of unknowns to IgSelect affinity ligand standards assayed at the same time.

Warranty: IgSelect 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 CaptureSelect Caustic stable human IgG ligand
* Standard solution: 100 µl CaptureSelect Caustic stable human IgG ligand
* Biotinylated reagents: 100 µl Biotinylated Goat IgG anti CaptureSelect Caustic stable human IgG 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
* *Human IgG (for protocol for samples containing IgG)*
* 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 for samples without human IgG

- 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 1:1000 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 5 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.

Note:
The assay is compatible with samples in one of the following elution buffers;
- PBS pH 2.0
- 0.1 M Glycine pH 2.5
- 0.1 M Acetic acid pH 2.9
- 0.1 M Phosphoric acid pH 1.5

When neutralized with 10 (v/v)% 1 M Tris pH 8.0, these buffers will not influence the sensitivity of the assay.

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.

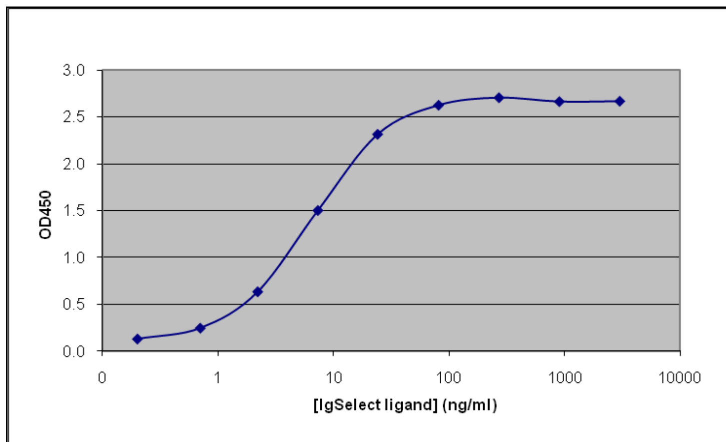

Figure 1: Calibration curve IgSelect leakage assay for samples without human IgG.

Protocol for samples containing human IgG

- 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.
- 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).
- Prepare a standard dilution serie by diluting the Standard solution. Recommended is 1000 times to 500 ng/ml in dilution buffer (2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4) containing half the concentration human IgG in comparison with the undiluted sample. Since the sample is diluted 1:1 with two times concentrated dilution buffer without IgG, the end concentration IgG in the samples and standard dilution serie are the same.

1: [500] = 1 µl Standard solution + 999 µl Human IgG and dilution buffer
2: [167] = 300 µl [500] + 600 µl Human IgG and dilution buffer
3: [56] = 300 µl [167] + 600 µl Human IgG and dilution buffer

Up to
8: [0.23] = 300 µl [0.69] + 600 µl Human IgG and dilution buffer

- 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 1:1000 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.
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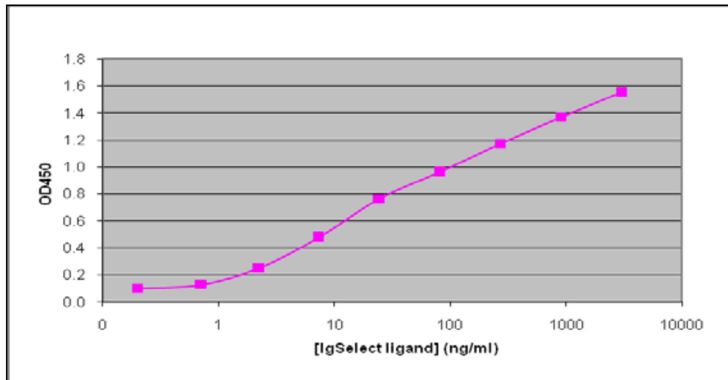

Figure 2: Calibration curve IgSelect leakage assay for samples with 1 mg/ml human IgG.

Contact

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