GENTAUR

DATA SHEET

Staphylococcus aureus Host Cell Proteins

Immunoenzymetric Assay for the Measurement of *Staphylococcus aureus* Host Cell Proteins Catalog # F320

Intended Use

This kit has been specifically developed and validated for use in detecting *Staphylococcus aureus* HCPs in Protalex's Protein A product. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

Summary and Explanation

Protein A as naturally produced by ***S. aureus*** has found many useful biological applications. It is widely used as an affinity ligand to purify antibodies and also found utility as a diagnostic and therapeutic agent. Even highly purified Protein A may contain detectable levels of other ***S. aureus*** proteins hereafter referred to as Host Cell Proteins (HCPs). When the Protein A is to be used for *in-vivo* diagnostic or therapeutic applications in humans and animals, HCP contamination must be reduced to the lowest levels practical to avoid adverse toxic or immunological reactions.

Immunological methods using antibodies to HCPs such as Western Blot and ELISA are widely accepted due to their specificity and sensitivity. While Western blot is a powerful method aiding in the identity of HCPs, it suffers from a number of limitations. Western blot is a complex and technique dependent procedure requiring a subjective interpretation of results. Furthermore, it is essentially a qualitative method and does not lend itself to obtaining quantitative answers. The sensitivity of Western blot is severely limited by the volume of sample that can be tested and by interference from the presence of high concentrations of the intended product. Western Blot may be able to detect HCPs in samples from upstream in the purification process but it often lacks adequate sensitivity to detect HCPs in purified downstream and final product. The microtiter plate immunoenzymetric assay (ELISA) method employed in this kit overcomes the limitations of Western blots providing on the order of 100 fold better sensitivity. This simple to use, highly sensitive, objective, and semi-quantitative ELISA is a powerful method to aid in optimal purification process development, process control, and in routine quality control and product release testing. This kit is "generic" in the sense that it is intended to react with essentially all of the HCPs that could contaminate the product independent of the purification process. The antibodies have been generated against and affinity purified using conditioned media to obtain HCPs typically encountered in initial product recovery steps. Because of the high sensitivity and broad reactivity of the antibodies, this generic kit has been successfully validated for testing of final product HCPs as well as in-process samples. The suitability of this kit for sample types not previously validated must be established experimentally by each laboratory.

Reagents & Materials Provided

Component	Product #
Anti-*S. aureus*:HRP Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative. 1x12mL	**F321**
Anti-*S. aureus* coated microtiter strips 12x8 well strips in a bag with desiccant	**F322**
***S. aureus* HCP Standards** Growth media derived HCPs in bovine albumin with preservative. Standards at 0, 5, 20, 75, 200, and 500ng/mL. 1 mL/vial	**F323**
Stop Solution 0.5N sulfuric acid. 1x12mL	**F006**
TMB Substrate 3,3',5,5' Tetramethyl benzidine. 1x12mL	**F005**
Wash Concentrate (20X) Tris buffered saline with preservative. 1x50mL	**F004**

Principle of the Procedure

The *Staphylococcus aureus* Host Cell Protein assay is a two-site immunoenzymetric assay utilizing a "reverse sequential protocol". In this protocol the samples, standards, and controls are first incubated in uncoated test tubes or small microfuge vials together with HRP enzyme labeled *S. aureus* antibodies for 1 hour. After this first incubation the reactant mixture is then transferred to anti-HCP antibody coated microtiter wells and incubated for another hour. The microtiter strips are then washed to remove any unbound reactants. The substrate tetramethyl benzidine (TMB) is then reacted for 30 minutes. After the addition of a "stop" reagent, the amount of hydrolyzed substrate is read on a microtiter plate spectrophotometer. The intensity of the colored substrate product will be directly proportional to the concentration of *S. aureus* proteins present in the sample.

Storage & Stability

* All reagents should be stored at 2°C to 8°C for stability until the expiration date printed.

* The substrate reagent should not be used if its stopped absorbance at 450nm is greater than 0.1.

* Reconstituted wash solution is stable until the expiration date of the kit.

Materials & Equipment Required But Not Provided

Microtiter plate reader spectrophotometer with dual wavelength capability at 450 & 650nm. *(If your plate reader does not provide dual wavelength analysis you may read at just the 450nm wavelength.)*
Pipettors – adjustable or fixed volume, at 100, 125, and 200µL
Repeating or multichannel pipettor - 100µL
Microtiter plate rotator (150 - 200 rpm)
Sample Diluent (recommended Cat # I028)
Distilled water
1 liter wash bottle for diluted wash solution

Precautions

* For Research or Manufacturing use only.

* Stop reagent is 0.5N H_2SO_4. Avoid contact with eyes, skin, and clothing. At the concentrations used in this kit, none of the other reagents are believed to be harmful.

* This kit should only be used by qualified technicians.

Preparation of Reagents

* Bring all reagents to room temperature.

* Dilute wash concentrate to 1 liter in distilled water, label with kit lot and expiration date, and store at 4°C.

Procedural Notes

1. Complete washing of the plates to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. We advise against the use of automated or other manual operated vacuum aspiration devices for washing plates as these may result in lower specific absorbances, higher non-specific absorbance, and more variable precision. The manual wash procedure described below generally provides lower backgrounds, higher specific absorbance, and better precision. If duplicate CVs are poor or if the absorbance of the 0 standard minus a substrate blank is greater than 0.200, evaluate plate washing procedure for proper performance.

2. High Dose Hook Effect may be observed in samples with very high concentrations of HCP. High Dose Hook Effect is due to insufficient excess of antibody for very high concentrations of HCPs present in the sample. Samples with greater than 250 µg/mL may give absorbances less than the 500 ng/mL standard. It is also possible for samples to have certain HCPs in concentrations exceeding the amount of antibody for that particular HCP. In such cases the absorbance of the undiluted sample may be lower than the highest standard in the kit however these samples will fail to show acceptable dilutional recovery/ parallelism as evidenced by an apparent increase in HCP concentration with increasing dilution. High Dose Hook is most likely to be encountered from samples early in the purification process. If a hook effect is possible, samples should also be assayed diluted. If the HCP concentration of the undiluted sample is less than the diluted sample this may be indicative of the hook effect. Such samples should be diluted at least to the minimum required dilution (MRD) where the dilution adjusted value remains essentially constant. The HCP value to be reported for such samples is the dilution corrected value at or greater than the established MRD. The diluent used should be compatible with accurate recovery. The preferred diluent is our Cat# I028 available in 100mL, 500mL, or 1 liter bottles. This is the same material used to prepare the kit standards. As the sample is diluted in I028 its matrix begins to approach that of the standards thus reducing any inaccuracies caused by dilutional artifacts. Other prospective diluents must be tested for recovery by using them to dilute the 500ng/mL standard, as described in the "Limitations" section below.

3. If the substrate has a distinct blue color prior to performing the assay it may have been contaminated. If this appears to be the case, read 100µL of substrate plus 100µl of stop against a water blank. If the absorbance is greater than 0.1 it may be necessary to obtain new substrate or the sensitivity of the assay may be compromised.

4. Strips should be read within 15 minutes after adding stop solution since color will fad over time.

Limitations

* Before relying exclusively on this assay to detect host cell proteins, each laboratory should validate that the kit antibodies and assay procedure yield acceptable specificity, accuracy, and precision. A suggested protocol for this validation can be obtained by contacting our Technical Services Department or at our web site.

* The standards used in this assay are comprised of *S. aureus* HCPs obtained from growth media. Western blot analysis of the antibodies used in this kit demonstrates that they recognize the majority of distinct PAGE separated bands seen using a sensitive protein staining method like silver stain or colloidal gold. This kit has been validated for HCP detection in sample types used in the purification procedure at the time of validation. If this kit is to be applied to other products or sample types, it must be validated to establish acceptable accuracy and specificity for those sample types.

* Certain sample matrices may interfere in this assay. The product protein itself, high or low pH, high salt, detergents, or other components in the sample matrix may result in either positive or negative interference in this assay. It is advised to test all sample matrices for interference by diluting the 500ng/mL standard, 1 part to 4 parts of the matrix containing no or very low HCP contaminants. This diluted standard when assayed as an unknown should give a value of 75 to 125 ng/mL. Consult ***Cygnus Technologies*** Technical Service Department for advice on how to quantitate the assay in problematic matrices.

Assay Protocol

* The assay is very robust such that assay variables like incubation times, sample size, and other sequential incubation schemes can be altered to manipulate assay performance for more sensitivity, increased upper analytical range, or reduced sample matrix interference. Before modifying the protocol from what is recommended, users are advised to contact our technical services for input on the best way to achieve your desired goals.

* The protocol specifies the use of an approved microtiter plate shaker or rotator for the immunological step. If you do not have such a device it is possible to incubate the plate without shaking, however it will be necessary to extend the immunological incubation step in the microtiter plate wells by about 30 minutes to one hour in order to achieve comparable results to the 1 hour shaking protocol. Do not shake during the 30 minute substrate incubation step as this may result in higher backgrounds and worse precision.

* Bring all reagents to room temperature.

* Set-up plate spectrophotometer to read dual wavelength at 450nm for the test wavelength and 650nm for the reference wavelength. We suggest blanking the instrument using one of the zero standard wells after assay completion.

* All standards, controls and test samples should be assayed at least in duplicate.

* Maintain a repetitive timing sequence from well to well for all assay steps to insure that all incubation times are the same for each well.

* Make a work list for each assay to identify the location of each standard, control and sample.

* Thorough washing is essential to proper performance of this assay. Automated plate washing systems or other vacuum aspiration devices are not recommended. The manual method described in the assay protocol is preferred for best precision, sensitivity and accuracy. A more detailed discussion of this procedure can be obtained from our Technical Services Department or on our web site.

Quality Control

* It is recommended that each laboratory assay appropriate quality control samples in each run to insure that all reagents and procedures are correct. **You are strongly urged to make controls in your typical sample matrix using HCPs derived from your cell line. These controls can be aliquoted into single use vials and stored frozen for long-term stability.**

* Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples greater than 10 ng/mL and < 500 ng/mL. CVs for samples < 10 ng/mL may be greater than 10%.

* For optimal performance the absorbance of the substrate when blanked against water should be < 0.1.

Assay Protocol

1. Use clean polypropylene test tubes or micro-centrifuge vials with caps.

2. Pipette 100µL of standards, (0-500ng/mL), controls, and samples into labeled tubes or vials as indicated on work list.

3. Pipette 200µL of anti-*S.aureus* HCP:HRP conjugate (#F321) into each tube or vial. *These volumes of 100µl for the sample and 200µL for the conjugate are recommended, assuming the assay is performed in duplicate. If assaying in triplicate or more, the relative volumes should be adjusted appropriately.

4. Cap, vortex, and allow to incubate for 1 hour at room temperature.

5. Transfer 125µL of the reaction mixture to duplicate coated wells in the anti-*S.aureus* HCP coated microtiter strips as indicated on the work list.

6. Cover or place into a zip-lock plastic bag. Transfer to rotator and incubate at ~ 180rpm for 1 hour at room temperature, 24°C ± 4°.

7. Dump contents of wells into waste or gently aspirate using a multi-channel pipettor. Blot and vigorously bang out residual liquid over low lint absorbent paper. Wash generously with diluted wash solution by flooding the wells with a squirt bottle or by pipetting ~350µL of wash solution. Dump and bang again. Repeat for a total of 4 washes. Wipe off any liquid from the bottom outside of the microtiter wells as residue can interfere in the reading step.

8. Pipette 100µL of TMB substrate (#F005) into all wells.

9. Place cover on plate and incubate for 30 minutes at room temperature. Do not shake!

10. Pipette 100µL of stop solution (#F006).

11. Read absorbance at 450/650nm blanking on the Zero standard.

Calculation of Results

The standards may be 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 point to point, cubic spline, or 4 parameter logistic fit. **Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies!** Data may also be manually reduced by plotting the absorbance values of the standard on the y-axis versus concentration on the

x-axis and drawing a smooth point to point line. Absorbances of samples are then interpolated from this standard curve.

Example Data

Well #	Contents	Abs. at 450nm	Mean Abs.	ng/mL HCP
1A	Zero Std	0.060		
1B	Zero Std	0.063	0.062	
1C	5ng/mL	0.117		
1D	5ng/mL	0.116	0.117	
1E	20ng/mL	0.320		
1F	20ng/mL	0.346	0.333	
1G	75ng/mL	0.968		
1H	75ng/mL	0.905	0.937	
2A	200ng/mL	2.029		
2B	200ng/mL	1.975	2.002	
2C	500ng/mL	3.567		
2D	500ng/mL	3.512	3.540	
2E	sample A	0.349		
2F	sample A	0.353	0.351	21.6
2G	sample B	0.957		
2H	sample B	0.914	0.936	75.2

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