Version 19-04/24

User's Manual

SP2/0 Host Cell Protein ELISA kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is intended for use in determining the presence of SP 2/0 host cell protein impurities in products manufactured by recombinant expression in SP 2/0 host cells.

Principles of Testing

The SP 2/0 assay is a two-site immunoenzymetric assay. Samples containing SP 2/0 HCPs are reacted in microtiter strips coated with an affinity purified capture antibody. A second horseradish peroxidase (HRP) enzyme labeled anti-SP 2/0 antibody is reacted, simultaneously resulting in the formation of a sandwich complex of solid phase antibody-HCP-enzyme labeled antibody. The microtiter strips are washed to remove any unbound reactants. The substrate, tetramethylbenzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and is directly proportional to the concentration of SP 2/0 HCPs present.

Reagents And Materials Provided

1. **Anti-SP 2/0 HRP** Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative. 1x12mL
2. **Anti-SP 2/0 coated microtiter strips** 12x8 well strips in a bag with desiccant.
3. **SP 2/0 HCP Standards** Solubilized SP 2/0 HCPs in human immunoglobulin with preservative. Standards at 0, 2, 8, 25, 75, and 200ng/mL. 1 mL/vial
4. **Stop Solution** 0.5M sulfuric acid. 1x12mL
5. **TMB Substrate** 3,3',5,5' Tetramethylbenzidine. 1x12mL
6. **Wash Concentrate (20X)** Tris buffered saline with preservative. 1x50mL

Materials Required But Not Supplied

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.)

1. Pipettors - 50µL and 100µL
2. Repeating or multichannel pipettor - 100µL
3. Microtiter plate rotator (400 - 600 rpm)
4. Sample Diluent recommended, Cat # CD183A
5. Distilled water
6. 1 liter wash bottle for diluted wash solution

Storage

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

After prolonged storage, you may notice a salt precipitate and/or yellowing of the wash concentrate. These changes will not impact assay performance. To dissolve the precipitate, mix the wash concentrate thoroughly and dilute as directed in the 'Reagent Preparation' section.

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

Reagent Preparation

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.

Assay Procedure

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 is greater than 0.200, evaluate plate washing procedure for proper performance.
2. High Dose Hook Effect or poor dilutional linearity 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 samples upstream in the purification process. Samples greater than 200µg/mL may give absorbances less than the 200 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 linearity/parallelism as evidenced by an apparent increase in dilution corrected HCP concentration with increasing dilution. High Dose Hook and poor dilutional linearity are 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 dilutions (MRDs) as established by your qualification studies using your actual final and in-process drug samples. The MRD is the first dilution at which all subsequent dilutions yield the same HCP value within the statistical limits of assay precision. 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# CD183A 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 CD183A, 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 200ng/mL standard, as described in the "Limitations" section.

Assay Protocol

The assay protocol is a simultaneous incubation of sample with HRP conjugated antibody. This yields a sensitivity of ~1ng/mL and requires 2.5 hours to complete.

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 use of an approved orbital microtiter plate shaker for the immunological steps. These can be purchased from most laboratory supply companies. 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 plate by about one hour in order to achieve comparable results to the routine protocols. 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.

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.

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

Maintain a repetitive timing sequence from well to well for all assay steps to ensure 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.

It is recommended that each laboratory assay appropriate quality control samples in each run to ensure 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.

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

Strips should be read within 30 minutes after adding stop solution since color will fade over time.

1. Pipette 50µL of standards, controls and samples into wells indicated on work list.
2. Pipette 100µL of anti-SP 2/0:HRP into each well.
3. Cover & incubate on orbital shaker at 400 – 600 rpm for 2 hours at room temperature, 24°C ± 4°C.
4. Dump contents of wells into waste. Blot and gently but firmly tap over absorbent paper to remove most of the residual liquid. Overly aggressive banging of the plate or use of vacuum aspiration devices in an attempt to remove all residual liquid is not necessary and may cause variable dissociation of antibody bound material resulting in lower residual ODs and worse precision. Fill wells generously to overflowing with diluted wash solution using a squirt bottle or by pipetting in ~350µL. Dump and tap again. Repeat for a total of 4 washes. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. Do not allow wash solution to remain in wells for longer than a few seconds. Do not allow wells to dry before adding substrate.
5. Pipette 100µL of TMB substrate.
6. Incubate at room temperature for 30 minutes. DO NOT SHAKE.
7. Pipette 100µL of Stop Solution.
8. Read absorbance at 450/650nm.

Quality Control

Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples greater than 2 ng/mL and less than 200 ng/mL. CVs for samples less than 2 ng/mL may be greater than 10%.

It is recommended that each laboratory assay appropriate quality control samples in each run to ensure that all reagents and procedures are correct.

Calculation

The standards may be used to construct a standard curve with values reported in ng/mL "total immunoreactive HCP equivalents" (See Limitations section). 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 450-650nm	Mean Abs.
A1	Zero Std	0.000	0.001
B1	Zero Std	0.002	
C1	2ng/mL	0.012	0.017
D1	2ng/mL	0.022	
E1	8ng/mL	0.059	0.056
F1	8ng/mL	0.053	
G1	25ng/mL	0.177	0.174
H1	25ng/mL	0.170	
A2	75ng/mL	0.540	0.540
B2	75ng/mL	0.539	
C2	200ng/mL	1.520	1.501
D2	200ng/mL	1.482	

Precision

Both intra (n=20 replicates) and inter-assay (n=5 assays) precision were determined on 3 pools with low (2ng/mL), medium (~25ng/mL), and high concentrations (~75ng/mL). The % CV is the standard deviation divided by the mean and multiplied by 100.

Intra-assay			Inter-assay		
# of tests	Mean ng/mL	%CV	# of assays	Mean ng/mL	%CV
20	2.0	15.6	5	2.0	19.1
20	25	1.6	5	25	5.4
20	75	1.8	5	75	6.3

Sensitivity

The lower limit of detection (LOD) is defined as that concentration corresponding to a signal two standard deviations above the mean of the zero standard. LOD is ~1 ng/mL.

The lower limit of quantitation (LOQ) is defined as the lowest concentration, where concentration coefficients of variation (CVs) are <20%. The LOQ is ~2 ng/mL.

Specificity

1D Western blot analysis against other cell lines of SP2/0 indicate that most of the proteins are conserved among all strains. Thus this assay should be useful for detecting HCP's from other SP2/0 cell lines. Each end user must qualify that this kit is adequately reactive and specific for their samples. 1D Western blot is highly orthogonal to ELISA and to non-specific protein staining methods such as silver stain or colloidal gold. As such, the lack of identity between silver stain and western blot does not necessarily mean there is no antibody to that protein or that the ELISA will not detect that protein. If you desire a much more sensitive and specific method than Western blot to detect the reactivity of the antibodies in this kit to your individual HCPs Cygnus Technologies is pleased to perform AAE as a service to provide coverage information of the antibodies to the HCPs in your process samples. This method has been shown to be much at least 100 fold more sensitive than Western blots in detecting antibody reactivity to individual HCPs. The same antibody as is used for both capture and HRP label can be purchased separately. Normal human IgG and mouse IgG were shown to be non-reactive in this assay at concentrations of

Interferences

Various buffer matrices have been evaluated by adding known amounts of SP2/0 HCP preparation used to make the standards in this kit. Because this assay is designed to minimize matrix interference most of these buffers yielded acceptable recovery defined as between 80-120%. While the standards used in this kit contain 4mg/mL normal human immunoglobulin, some product human IgG particularly at very high concentrations (>5mg/mL) may interfere in the accurate measurement of SP2/0 HCP's. In general extremes in pH (8.5), high salt concentration, and certain detergents can cause under-recovery. Each user should verify that their sample matrices yield accurate recovery. Such an experiment can be performed, by diluting the 200ng/mL standard provided with this kit, into the sample matrix in question as previously described in the "Limitations" section.

Hook Capacity

Increasing concentrations of HCPs> 200 ng/mL were assayed as unknowns. The hook capacity, defined as that concentration which will give an absorbance reading less than the 200 ng/mL standard was>200µg/mL.

Precautions

1. For Research or Manufacturing use only.
2. Stop reagent is 0.5M H_2SO_4. Avoid contact with eyes, skin, and clothing.
3. The standards used in this kit contain human immunoglobulin derived from human serum. The source material was tested and found negative for antibody to HIV and Hepatitis B surface antigen. No known method can offer total assurance that HIV or Hepatitis virus or other infectious agents are absent, therefore handle these reagents as if they are potentially infectious.
4. This kit should only be used by qualified technicians.