PG13 Host Cell Proteins

Immunoenzymetric Assay for the Measurement of PG13 Host Cell Proteins Catalog # F995

Intended Use

This kit is intended for use in determining the presence of host cell protein impurities in products manufactured by expression in PG13 cells. The kit is for **Research and Manufacturing Use Only** and is not intended for diagnostic use in humans or animals.

Summary and Explanation

Retroviral packaging in PG13 cells is a cost-effective way to produce a high titer of retrovirus capable of infecting human cells for gene and cell therapies. The manufacturing and purification process of these products leaves the potential for impurities by host cell proteins (HCPs) from PG13 cells. Such impurities can reduce the efficacy of the therapeutic agent and result in adverse toxic or immunological reactions and thus it is desirable to reduce HCP impurities to the lowest levels practical.

This simple to use, objective, and semi-quantitative ELISA is a sensitive and specific 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 a mild lysate of PG13 cells. The antibodies used in this kit were characterized by Antibody Affinity Extraction (AAE) and Mass Spectrometry, demonstrating reactivity to the majority of HCPs.

Special procedures were utilized in the generation of these antibodies to ensure that low molecular weight and less immunogenic impurities as well as high molecular weight components would be represented. As such, this kit can be used as a process development tool to monitor the optimal removal of host cell impurities as well as in routine final product release.

This highly sensitive ELISA kit was qualified for testing of final product HCPs by using actual in-process and final drug substance samples. Each user of this kit is encouraged to perform a similar qualification study to demonstrate it meets their analytical needs. The suitability of this kit for a given sample type and product must be determined and qualified experimentally by each laboratory. If you deem a more process specific assay is necessary, Cygnus Technologies is available to apply its proven technologies to develop such antibodies and assays on a custom basis.

Principle of the Procedure

The PG13 assay is a two-site immunoenzymatic assay. Samples containing PG13 HCPs are reacted simultaneously with a horseradish peroxidase (HRP) enzyme labeled anti-PG13 antibody (goat polyclonal) in microtiter strips coated with an affinity purified capture anti-PG13 antibody (goat polyclonal). The immunological reactions result 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 PG13 HCPs present.

Reagents & Materials Provided

Component	Product #
Anti-PG13:HRP Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative. 1x12mL	**F996**
Anti-PG13 coated microtiter strips 12x8 well strips in a bag with desiccant	**F997**
PG13 HCP Standards PG13 HCPs in bovine serum albumin with preservative. Standards at 0, 3, 6, 12, 25, 50, 100 and 200ng/mL. 1 mL/vial	**F998**
Stop Solution 0.5M sulfuric acid. 1x12mL	**F006**
TMB Substrate 3,3',5,5' Tetramethylbenzidine. 1x12mL	**F005**
Wash Concentrate (20X) Tris buffered saline with preservative. 1x50mL	**F004**

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 - 50µL and 100µL
- Repeating or multichannel pipettor - 100µL
- Microtiter plate rotator (400-600 rpm)
- Sample Diluent (recommended Cat # I028)
- Distilled water
- 1 liter wash bottle for diluted wash solution

Storage & Stability

- 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 'Preparation of Reagents' section. Reconstituted wash solution is stable until the expiration date of the kit.

Precautions

- For Research or Manufacturing use only.
- Stop reagent is 0.5M H_2SO_4. Avoid contact with eyes, skin, and clothing.
- This kit should only be used by qualified technicians.

Preparation of Reagents

- Bring all reagents to room temperature.
- Dilute 20x wash concentrate to 1x in 1 liter of distilled water, label with kit lot and expiration date, and store at 4°C.

Procedural Notes

- Complete washing of the plates to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. The manual wash procedure described below generally provides lower backgrounds, higher specific absorbance, and better precision than automated plate washers. If duplicate CVs are poor, or if the absorbance of the '0' standard is greater than 0.300, evaluate plate washing procedure for proper performance.

Limitations

- Before relying exclusively on this assay to detect host cell proteins, each laboratory should qualify that the kit antibodies and assay procedure yield acceptable specificity, accuracy, and precision. A suggested protocol for this qualification can be obtained from our Technical Services Department or our web site.
- The standards used in this assay are comprised of PG13 HCPs solubilized by mechanical disruption and detergent. AAE analysis of the antibodies used in this kit demonstrates that they recognize the majority of distinct PAGE separated bands seen using silver staining of protein.
- Certain sample matrices may interfere in this assay. The standards used in this kit attempt to simulate typical sample protein and matrices. However, the potential exists that the product itself or other components in the sample matrix may result in either positive or negative interference in this assay. High or low pH, detergents, urea, high salt concentrations, and organic solvents are some of the known interference factors. It is advised to test all sample matrices for interference by diluting the 200ng/mL standard 1 part to 4 parts of the matrix containing no or very low HCP impurities. This diluted standard, when assayed as an unknown, should give an added HCP value in the range of 32 to 48ng/mL. Consult Cygnus Technologies' Technical Service Department for advice on how to quantitate the assay in problematic matrices.
- Avoid the assay of samples containing sodium-azide (NaN3) which will destroy the HRP activity of the conjugate and could result in the under-estimation of HCP levels.

Assay Protocol

- 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 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.
- Thorough washing is essential to proper performance of this assay. 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. In addition, a video demonstration of proper plate washing technique is available in the 'Technical Resources' section of our web site.
- 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 your 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.

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

Assay Protocol

1. Pipette 100µL of anti-PG13:HRP (#F996) into each well.

2. Pipette 50µL of standards (#F998), controls, and samples into wells indicated on work list.

3. Cover & incubate on orbital shaker at 400 - 600rpm 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 to remove all residual liquid is not necessary and may cause variable dissociation of antibody bound material resulting in lower 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 (#F005).

6. Incubate at room temperature for 30 minutes. DO NOT SHAKE.

7. Pipette 100µL of Stop Solution (#F006).

8. Read absorbance at 450/650nm.

Example Data

Well #	Contents	Abs. at 450-650nm	Mean Abs.
H1	Zero Std	0.074	0.076
H2	Zero Std	0.078	
G1	3ng/mL	0.124	0.121
G2	3ng/mL	0.119	
F1	6ng/mL	0.174	0.175
F2	6ng/mL	0.176	
E1	12ng/mL	0.271	0.268
E2	12ng/mL	0.265	
D1	25ng/mL	0.496	0.486
D2	25ng/mL	0.477	
C1	50ng/mL	0.840	0.838
C2	50ng/mL	0.836	
B1	100ng/mL	1.520	1.513
B2	100ng/mL	1.506	
A1	200ng/mL	2.751	2.754
A2	200ng/mL	2.756	

Calculation of Results

The standards may be used to construct a standard curve with values reported in ng/mL "total immuno-reactive HCP equivalents". 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.

Quality Control

- Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples in the range of 6-200ng/mL. CVs for samples less than 6ng/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.

Performance Characteristics

Cygnus Technologies has qualified this assay by conventional criteria as indicated below. A copy of this qualification report can be requested on our web site by searching "Request a Qualification Summary". This qualification is generic in nature and is intended to supplement but not replace certain user and product specific qualification and qualification that should be performed by each laboratory. At a minimum, each laboratory is urged to perform a spike and recovery study in their sample types. In addition, any of your sample types containing process derived HCPs within or above the analytical range of this assay should be evaluated for dilutional linearity to ensure that the assay is accurate and has sufficient antibody excess for your particular HCPs. Each laboratory and technician should also demonstrate competency in the assay by performing a precision study similar to that described below. A more detailed discussion of recommended user qualification protocols can be requested by contacting our Technical Services Department or on our web site.

Sensitivity

The lower limit of detection (**LOD**) is defined as that concentration corresponding to a signal three standard deviations above the mean of the zero standard. LOD is ~0.4 ng/mL.

The lower limit of quantitation (**LLOQ**) is defined as the lowest concentration where concentration coefficients of variation (CVs) are less than 20%. The LLOQ is ~3 ng/mL.

Specificity/Cross-Reactivity

Cross reactivity to non-HCP components has not been extensively investigated with this kit. You should evaluate components in your samples for positive interferences such as cross reactivity and non-specific binding. Negative interference studies are described below.

Precision

Both intra-assay (n=20 replicates) and inter-assay (n=10 assays) precision were determined on 4 pools with low (~3.8 ng/mL), low-medium (~20 ng/mL), medium (~75 ng/mL), and high concentrations (~150 ng/mL). The % CV is the standard deviation divided by the mean and multiplied by 100.

Pool	Intra assay CV	Inter assay CV
Low	8.4%	6.5%
Low-medium	2.4%	2.4%
Medium	2.4%	3.2%
High	2.2%	2.9%

Recovery/Interference Studies

In-process and final formulation drug substances were evaluated by adding known amounts of PG13 HCP preparation used to make the standards in this kit. All of these samples yielded acceptable recovery defined as between 80-120%. The standards used in this kit contain 8mg/mL of bovine serum albumin intended to simulate non-specific protein effects of most sample proteins. However, very high concentrations of some products may interfere in the accurate measurement of HCPs. In general, extremes in pH (less than 5.0 and greater than 8.5), high salt concentration, high polysaccharide concentrations, urea, organic solvents, and most detergents can cause under-recovery. Each user should qualify 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 described in the "Limitations" section.

Ordering Information/ Customer Service

Cygnus Technologies also offers kits for the extraction and detection of Host Cell DNA. The following kits are available:

- Residual Host Cell DNA extraction:

  Cat # D100W, DNA Extraction Kit in 96 deep well plate
  Cat # D100T, DNA Extraction Kit in microfuge tubes

To place an order or to obtain additional product information contact *Cygnus Technologies*:

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