HEK 293 HCP Assay Reagent Set for Gyrolab®

Catalog # G650S-1

Intended Use

This Reagent Set is intended for use in determining the presence of host cell protein contamination in products manufactured by expression in HEK 293 cell lines. The Reagent Set is for **Research and Manufacturing Use Only** and is not intended for diagnostic use in humans or animals. **This Reagent Set is designed to be used in conjunction with a Gyrolab® Bioaffy™ 4000 HC Assay Toolbox which should be purchased separately from Gyros Protein Technologies (Product Number P0020852).**

Summary and Explanation

This easy to use, high throughput, and semi-quantitative assay is a method to aid in optimal purification process development, process control, routine quality control, and product release testing. It is derived from the same antibodies and antigen used in the Cygnus HEK 293 HCP Re-supply ELISA kit, Cat# F650S. The reagents are generic in the sense that they are intended to react with essentially all the host cell proteins (HCPs) that could contaminate the product independent of the purification process. The antibodies have been generated in goats and rabbits and antigen affinity purified using HEK 293 HCPs found in protein-free conditioned media.

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

Each user of this Reagent Set is encouraged to perform a qualification study to demonstrate that it meets their analytical needs. Provided this Reagent Set can be satisfactorily qualified for your samples, the application of a more process specific assay may not be necessary. However, if your qualification studies indicate the antibodies in this set are not sufficiently reactive with your process specific HCPs, it may be desirable to also develop a more process specific assay. Cygnus Technologies is available to apply its proven technologies to develop process specific antibodies and assays on a custom basis.

Principle of the Procedure

This HEK 293 HCP assay is a three-step sandwich immunoassay that has been developed on the Gyrolab platform. The biotinylated capture antibody is introduced into microstructures in a Gyrolab Bioaffy CD to saturate capture columns packed with porous beads that are coupled with streptavidin. Subsequently, samples containing HEK 293 HCP are volume defined and introduced into the microstructures where HEK 293 HCP antigens are captured on the capture column. Finally, a detecting reagent labeled with a suitable fluorophore is added. The integrated fluorescent signal represents the collective response from the HEK 293 HCP reaction. The assay run time is approximately 100 minutes.

Reagents & Materials Provided

Component	Product #
Anti-HEK 293 HCP Capture Affinity purified goat and rabbit antibody conjugated to biotin in a protein matrix with preservative. 60 µL	**G654S-1**
Anti-HEK 293 HCP Detection Affinity purified rabbit antibody conjugated to a fluorescent probe in a protein matrix with preservative. 60 µL	**G655S-1**
HEK 293 HCP Antigen Concentrate HEK HCPs in a protein matrix with preservative. 100 µg/mL x 60 µL	**G656S-1**

Materials & Equipment Required But Not Provided

- Gyrolab instrument
- Gyrolab Bioaffy 4000 HC Assay Toolbox (Gyros Protein Technology Product Number P0020852)
- Pipettes – 10 µL through 200 µL
- Multichannel pipette – 1 µL – 50 µL for liquid transfer from tubes to plate
- Dilution tubes (96-tube rack)
- 1X PBS + 0.01% Tween-20

Storage & Stability

- Antigen Concentrate and HCP Capture antibody should be stored at -20°C.
- HCP Detection antibody should be stored at 2°C to 8°C for stability until the expiration date printed on the kit.

Precautions

- For Research or Manufacturing use only.
- This Reagent Set should only be used by qualified technicians.
- Technicians must be adequately trained and qualified on the operation and maintenance of the Gyrolab instrument before using this Reagent Set.
- This Reagent Set lot has been qualified and released using the specific lots of components in the set. Do not mix Reagent Set components from different lots.
- This Reagent Set has been specially designed to be compatible with the Gyrolab Bioaffy 4000 HC Assay Toolbox. Use of other components may result in irregular or unexpected results.

Preparation of Reagents

- Bring all reagents and the Gyrolab Bioaffy 4000 HC Assay Toolbox to room temperature.
- Prepare 1 liter of 1X PBS with 0.01% Tween-20.
- Prepare 1 liter of Gyrolab Wash Buffer pH 11

Procedural Notes

- Refer to Instruction for Use for the Gyrolab Bioaffy 4000 HC Assay Toolbox.
- It is recommended that customers spin down all materials provided in this reagent set.
- **The Minimum Required Dilution (MRD) must be determined for all sample types used in this assay.** Poor dilution linearity may be observed in samples with very high concentrations of HCP. It is possible for samples to have certain HCPs in concentrations exceeding the amount of antibody for that particular HCP. In such cases the signal of the sample at all dilutions may be lower than the highest standard using the Reagent Set, however these samples will fail to show acceptable dilution linearity as evidenced by an apparent increase in dilution corrected HCP concentration with increasing dilution. Samples should be diluted at least to the 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 back-calculated 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.

Limitations

- Before relying exclusively on this assay to detect HCPs, each laboratory should qualify that the Reagent Set antibodies and assay procedure yield the acceptable specificity, accuracy, and precision. A suggested protocol for this qualification can be obtained from our Technical Services Department or web site.
- The antigen concentrate used in this assay is comprised of HEK 293 HCPs obtained after the culture of null HEK 293 cells in protein free culture media. Because the majority of HCPs will show antigenic conservation among all strains of HEK, this Reagent Set should be adequately reactive to the vast majority of HCPs from your transfected cell line. However, there can be no guarantee that this assay will detect all proteins from your process.
- Certain sample matrices may interfere in this assay. The antigen concentrate used in this Reagent Set attempts 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 antigen concentrate in a matrix, containing no or very low HCP contaminants. This diluted antigen when assayed as an unknown should return the original concentration once corrected for dilution.

Assay Protocol

- Both the HEK 293 HCP detection antibody, Product #G655S-1 and the Gyrolab Bioaffy 4000 HC CD are light sensitive. Minimize the exposure of these components to light.
- Cygnus has established a valid analytical range of 4 - 8,000 ng/mL. You can determine your standard curve range based on your analytical needs.
- 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 should be aliquoted into single use vials and stored frozen for long-term stability.

Tube #	Dilute from	HCP Sample Dilution Buffer	Final Concentration	Plate Position
1	Stock	N/A	100 µg/mL	N/A
2	20 µL of Tube 1	180 µL	10,000 ng/mL	N/A
3	80 µL of Tube 2	20 µL	8,000 ng/mL	A2
4	25 µL of Tube 3	75 µL	2000 ng/mL	A3
5	25 µL of Tube 4	75 µL	500 ng/mL	A4
6	25 µL of Tube 5	75 µL	125 ng/mL	A5
7	25 µL of Tube 6	75 µL	31 ng/mL	A6
8	25 µL of Tube 7	75 µL	8 ng/mL	A7
9	25 µL of Tube 8	25 µL	4 ng/mL	A8
10	0 µL	50 µL	0 ng/mL	A1

* Tubes 3-10 (highlighted in green) represent the standard curve used for this Reagent Set.

Assay Protocol

1. Prime the Gyrolab instrument.

2. Using the Gyrolab HCP Sample Dilution Buffer from the Gyrolab Bioaffy 4000 HC Assay Toolbox, dilute the HEK 293 Antigen Concentrate, #G656S-1 according to the table above.

3. Using the Gyrolab HCP Sample Dilution Buffer from the Toolbox prepare multiple dilutions of each sample.

4. Place standards, controls, samples, Capture Antibody, #G654S-1, Detection Antibody, #G655S-1 and wash buffers in the 96-well microplate according to the loading list.

5. Load the microtiter plate and Gyrolab Bioaffy 4000 HC CD onto the Gyrolab instrument.

Quality Control

- Precision on duplicate samples should yield average % coefficients of variation of less than 20%.
- It is recommended that each laboratory assay appropriate quality control samples in each run to ensure that all reagents and procedures are correct.

Calculation of Results

The data should be evaluated in the Gyrolab Evaluator software module. Open the run and select 'Quantification'. In 'Analysis Setup' these settings are recommended:

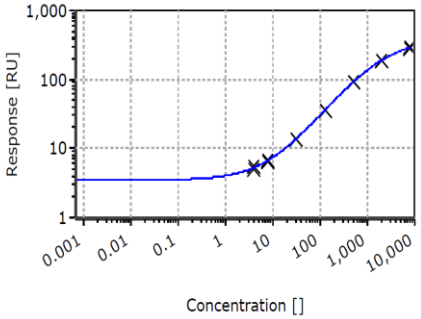
- Five parameter logistic curve
- Weight on response
- Limit of detection factor: 2

Example Data

G650S-1 Standard Curve on Gyrolab

Standard Concentration (ng/mL)	Mean RU	Std. Dev	% CV
0	3.5	0.29	6.4
4	4.7	0.20	5.2
8	5.6	0.18	2.0
31	12	0.05	1.5
125	32	1.14	3.6
500	88	0.25	2.2
2000	191	3.68	1.9
8000	297	5.66	1.8

Standard Curve Graph



Performance Characteristics

Cygnus has qualified this assay by conventional criteria as indicated below. This qualification is generic in nature and is intended to supplement but not replace certain user and product specific qualification and validation 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 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 obtained on our web site.

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 ~ 3 ng/mL.

Lower Limit of Quantitation **(LLOQ)** is defined as the lowest concentration for which the CV is typically <20%. The %CV for 8 replicates of the 4 ng/mL standard was 12%. We conservatively claim the LLOQ as ~4 ng/mL.

Specificity/Cross-Reactivity

Most of the proteins are conserved among all HEK lines. Therefore, this assay should be useful for detecting HCPs from other HEK 293 strains.

Cross-reactivity to non-HCP components has not been extensively investigated with this Reagent Set. You should evaluate components in your samples for positive interferences such as cross-reactivity and non-specific binding.

Precision

Both intra (n=20 replicates) and inter-assay (n=10 assays) precision were determined on 4 controls with low (~5 ng/mL), medium (~50 ng/mL and ~200 ng/mL), and high (~1000 ng/mL) HCP concentrations. The % CV is the standard deviation divided by the mean and multiplied by 100.

Control	Intra assay CV	Inter assay CV
5 ng/mL	15.6%	10.1%
50 ng/mL	6.5%	5.1%
200 ng/mL	4.1%	6.3%
1,000 ng/mL	9.3%	5.0%

Accuracy

The accuracy of the assay was determined by testing a low (~5 ng/mL), two medium (~50 ng/mL and ~200 ng/mL) and a high (~1000 ng/mL) control and determining the percent nominal for each sample. Percentage Nominal was calculated as (actual result divided by the theoretical result) x 100.

Control	% Nominal
5 ng/mL	103%
50 ng/mL	103%
200 ng/mL	101%
1,000 ng/mL	108%

Recovery/ Interference Studies

Various buffer matrices commonly used in purification of therapeutic proteins and monoclonal antibodies as well as in-process and final formulation drug substances were evaluated by adding known amounts of HEK 293 HCP preparation used to make the standards in this Reagent Set. All samples yielded acceptable recovery defined as between 80-120%. Very high concentrations of some products may interfere with the accurate measurement of HCPs. Each user should validate that their sample matrices yield accurate recovery.

Ordering Information/ Customer Service

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

Cygnus Cat# **G650S-1** is designed to be used in conjunction with Gyros Protein Technologies Cat. **P0020852**

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Email for Technical Support:
Reagent Set troubleshooting:
techsupport@cygnustechnologies.com

Toolbox and instrument troubleshooting:
support.europe@gyrosproteintech.com
support.northamerica@gyrosproteintech.com

To obtain additional product information on Gyrolab systems and materials, contact Gyros Protein Technologies:
www.gyrosproteintechnologies.com/gyrolab-immunoassay-solutions

For product information on Gyrolab Bioaffy 4000 HC Assay Toolbox:

www.gyrosproteintechnologies.com/immunoassays/products/gyrolab-bioaffy-4000-hc-assay-toolbox