# *E. coli* HCP 2G Assay Reagent Set for Gyrolab®

## Catalog # G1020

## Intended Use

This Reagent Set is intended for use in determining the presence of *E. coli* host cell protein contamination in products manufactured by recombinant expression in *E. coli*. 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™ 1000 HC Assay Toolbox which should be purchased separately from Gyros Protein Technologies (Product Number P0020667).**

## Summary and Explanation

Recombinant expression in *E. coli* is a relatively simple and cost-effective method for production of certain proteins and pDNA. Many of these products are intended for use as therapeutic agents in humans and animals, and as such, must be highly purified. The manufacturing and purification process of these products leaves the potential for contamination by host cell proteins (HCPs) from *E. coli.* Such contamination can reduce the efficacy of the therapeutic agent and result in adverse toxic or immunological reactions and thus it is desirable to reduce HCP contamination to the lowest levels practical.

This simple to use, highly sensitive, objective, and semi-quantitative immunoassay is a powerful method to aid in optimal purification process development, process control, 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 blended lysate of *E. coli* from a variety of strains. This relatively mild lysing procedure is intended to obtain HCPs typically encountered in initial product recovery steps, such as clarification of conditioned media when the product is secreted, or after osmotic shock or mild detergent and mechanical disruption, to obtain inclusion bodies and other intracellular proteins. The antibodies used in this kit were characterized by Antibody Affinity Extraction 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 contaminants 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 contaminants as well as in routine final product release testing.

Because of the high sensitivity and broad reactivity of the antibodies, this generic kit has been successfully qualified for testing of final product HCPs in many different products regardless of growth and purification process. 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 custom basis.

## Principle of the Procedure

This *E. coli* 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 *E. coli* HCP are volume defined and introduced into the microstructures where *E. coli* 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 *E. coli* HCP reaction. The assay run time is approximately 80 minutes.

## Reagents & Materials Provided

| Component | Product # |
|---|---|
| **Anti-*E. coli* HCP Capture**<br>Affinity purified goat antibody conjugated to biotin in a protein matrix with preservative. 60 µL | **G1021** |
| **Anti-*E. coli* HCP Detection**<br>Affinity purified goat antibody conjugated to a fluorescent probe in a protein matrix with preservative. 60 µL | **G1022** |
| ***E. coli* HCP Antigen Concentrate**<br>E. *coli* HCPs in a protein matrix with preservative. 100 µg/mL x 60 µL | **G1023** |

## Storage & Stability

- Store all components at -20°C.

## Materials & Equipment Required But Not Provided

- Gyrolab instrument
- Gyrolab Bioaffy 1000 HC Assay Toolbox (Gyros Protein Technology Product Number P0020667)
- Pipettes – 10 µL through 200 µL
- Multichannel pipette – 1 µL – 10 µL for liquid transfer from tubes to plate
- Dilution tubes (96-tube rack)
- 1X PBS + 0.01% Tween-20

## 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 1000 HC Assay Toolbox. Use of other components may result in irregular or unexpected results.

## Preparation of Reagents

- Bring all reagents and the Gyrolab Bioaffy 1000 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

1. Refer to Instruction for Use for the Gyrolab Bioaffy 1000 HC Assay Toolbox.

2. It is recommended that customers pulse vortex and spin down all materials provided in this reagent set.

3. **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 *E. coli* HCPs obtained after the culture of *E. coli* cells in protein free culture media. Because the majority of HCPs will show antigenic conservation among all strains of *E. coli*, 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 *E. coli* HCP detection antibody, Product #G1022, and the Gyrolab Bioaffy 1000 HC CD are light sensitive. Minimize the exposure of these components to light.
- Cygnus has established a valid analytical range of 5 - 3,000 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 | 60 µL of Tube 2 | 140 µL | 3,000 ng/mL | **A2** |
| 4 | 60 µL of Tube 3 | 120 µL | 1,000 ng/mL | **A3** |
| 5 | 50 µL of Tube 4 | 150 µL | 250 ng/mL | **A4** |
| 6 | 100 µL of Tube 5 | 100 µL | 125 ng/mL | **A5** |
| 7 | 80 µL of Tube 6 | 120 µL | 50 ng/mL | **A6** |
| 8 | 40 µL of Tube 7 | 160 µL | 10 ng/mL | **A7** |
| 9 | 100 µL of Tube 8 | 100 µL | 5 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. Refer to Gyros user guide for Gyrolab Bioaffy 1000 HC Assay Toolbox for assay set-up and instrument priming.**

**2. Using the Gyrolab HCP Sample Dilution Buffer from the Gyrolab Bioaffy 1000 HC Assay Toolbox, dilute the *E. coli* Antigen Concentrate, #G1023 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, #G1021, Detection Antibody, #G1022 and wash buffers in the 96-well microplate according to the loading list.**

**5. Load the microtiter plate and Gyrolab Bioaffy 1000 HC CD onto the Gyrolab instrument.**

## 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

## 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.

## Example Data Set From Live Run

| G1020 Standard Curve | | | |
|---|---|---|---|
| **Standard Concentration (ng/mL)** | **Mean RU** | **STD. DEV.** | **% CV** |
| **0** | **1.9** | **0.07** | **4.3** |
| **5** | **2.4** | **0.07** | **3.6** |
| **10** | **3.2** | **0.12** | **4.7** |
| **50** | **8.7** | **0.36** | **5.2** |
| **125** | **19** | **0.57** | **3.6** |
| **250** | **34** | **1.2** | **4.3** |
| **1,000** | **112** | **2.4** | **2.6** |
| **3,000** | **246** | **8.0** | **4.1** |

## 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 ~ 3.5 ng/mL.

Lower Limit of Quantitation **(LLOQ)** is defined as the lowest concentration for which the CV and nominal value is typically +/- 25%. The %CV for 8 replicates of the 5 ng/mL standard was 13% and the nominal recovery was 100%. We experimentally determined the LLOQ as ~5ng/mL in assay diluent.

### Precision

Both intra (n=20 replicates) and inter-assay (n=10 assays) precision were determined on 4 controls with low (~6.25 ng/mL), middle-1 (~75 ng/mL), middle-2 (~750 ng/mL), and high concentrations (~2,250 ng/mL). The % CV is the standard deviation divided by the mean and multiplied by 100.

| Pool | Intra assay CV | Inter assay CV |
|---|---|---|
| Low | 17.8% | 8.4% |
| Middle-1 | 5.2% | 4.0% |
| Middle-2 | 6.0% | 3.6% |
| High | 5.7% | 4.7% |

### Specificity/Cross-Reactivity

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

Cross reactivity 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.

### 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 *E. coli* 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 in 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*:

www.cygnustechnologies.com
Cygnus Technologies, LLC
1523 Olde Waterford Way
Leland, NC 28451 USA
Tel: +1 910-454-9442

Email for all Order inquiries:
orders@cygnustechnologies.com
order@gyrosproteintech.com
order.northamerica@gyrosproteintech.com

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 1000 HC Assay Toolbox:
www.gyrosproteintechnologies.com/gyrolab-bioaffy-1000-hc-assay-toolbox