

EndonucleaseGTP[®] Assay Reagent Set for Gyrolab[®]

Catalog # G960

Intended Use

This Reagent Set is intended for use in determining the presence of endonuclease impurities in recombinant vaccines and viral vector products. 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 Gyrolab[®] Bioaffy™ 1000 HC Assay Toolbox which should be purchased separately from Gyros Protein Technologies (Product Number P0020667).**

Summary and Explanation

Expression of recombinant viral vectors and vaccines in mammalian cell culture is a cost-effective method for production of commercial quantities of these novel biological drugs. The manufacturing and purification process of these products leaves the potential for impurities by HCPs and other additives such as enzymes used to remove endogenous DNA, RNA, and plasmid DNA used for viral vector production. 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 and added enzyme impurities to the lowest levels practical.

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 EndonucleaseGTP[®] ELISA kit, Cat# F960. The reagents are generic in the sense that they are intended to monitor the optimal removal of endonuclease impurities that could contaminate the product during the purification process. The antibodies have been generated against affinity purified endonuclease. This highly sensitive Reagent Set, developed with these antibodies, is accurate, sensitive, and precise for detection of genetically engineered endonuclease from *Serratia marcescens* such as Benzonase[®] Nuclease and DENARASE[®] in samples throughout the purification process.

Each user of this Reagent Set is encouraged to perform a qualification study to demonstrate that it meets their analytical needs.

Principle of the Procedure

This endonuclease 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 endonuclease impurities are volume defined and introduced into the microstructures where endonuclease 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 endonuclease reaction impurities. The assay run time is approximately 80 minutes.

Reagents & Materials Provided

Component	Product #
Anti-EndonucleaseGTP[®]: Capture Affinity purified goat antibody conjugated to biotin in a protein matrix with preservative. 60 µL	G961
Anti-EndonucleaseGTP[®]: Detection Affinity purified goat antibody conjugated to a fluorescent probe in a protein matrix with preservative. 60 µL	G962
EndonucleaseGTP[®]: Standard Endonuclease in a protein matrix with preservative. 100 µg/mL x 60 µL	G963

Storage & Stability

- Store all components at -20°C.

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.

Materials & Equipment Required But Not Provided

- Gyrolab instrument
- Gyrolab Bioaffy 1000 HC Assay Toolbox (Gyros Protein Technology Product Number P0020667)
- Pipettes – 10 µL through 1000 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

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 (included in the Gyrolab Bioaffy 1000 HC Assay Toolbox).

Procedural Notes

1. Refer to Instruction for Use for the Gyrolab Bioaffy1000 HC Assay Toolbox.
2. It is recommended to spin down all materials provided in this reagent set for 5 seconds.

Limitations

- Before relying exclusively on this assay to detect endonuclease impurities, 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 standard used in this assay is comprised of a known concentration of endonuclease.
- Certain sample matrices may interfere in this assay. The endonuclease standard 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 endonuclease standard in a matrix, containing no or very low endonuclease impurities. This diluted standard when assayed as an unknown should return the original concentration once corrected for dilution.

Assay Protocol

- Both the Anti-EndonucleaseGTP[®] detection antibody, Product #G962 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 0.05 - 200 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 endonucleases that are added during your process. 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	10 µL of Tube 1	990 µL	1,000 ng/mL	N/A
3	20 µL of Tube 2	80 µL	200 ng/mL	A2
4	25 µL of Tube 3	75 µL	50 ng/mL	A3
5	25 µL of Tube 4	75 µL	12.5 ng/mL	A4
6	25 µL of Tube 5	75 µL	3.1 ng/mL	A5
7	25 µL of Tube 6	75 µL	0.8 ng/mL	A6
8	25 µL of Tube 7	75 µL	0.2 ng/mL	A7
9	25 µL of Tube 8	75 µL	0.05 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 1000 HC Assay Toolbox, dilute the EndonucleaseGTP® Standard, #G963 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, #G961, Detection Antibody, #G962 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

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:

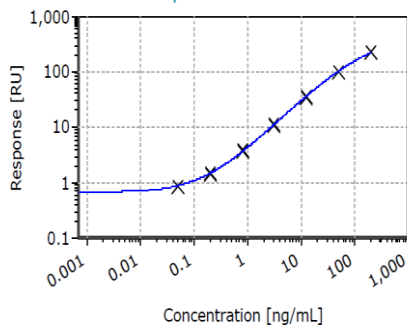
- Five parameter logistic curve
- Weight on response
- Limit of detection factor: 2

Example Data

G960 Standard Curve

Standard Concentration (ng/mL)	Response (RU)	Mean RU	% CV
0	0.706	0.693	2.7
	0.680		
0.05	0.875	0.858	2.9
	0.840		
0.2	1.44	1.49	4.0
	1.53		
0.8	3.65	3.76	4.0
	3.86		
3.1	11.2	11.4	2.5
	11.6		
12.5	35.3	36.1	3.0
	36.9		
50	104	102	2.3
	100		
200	232	229	1.9
	226		

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 endonucleases 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 purification process. Each laboratory and technician should also demonstrate competency in the assay by performing a precision study like the one 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 ~ 0.01 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 0.05 ng/mL standard was 19% and the nominal recovery was 109%. We experimentally determined the LLOQ as ~0.05ng/mL in assay diluent.

Specificity/Cross-Reactivity

Cross reactivity to non-endonuclease 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 (~0.5 ng/mL), medium (~5 ng/mL and 25 ng/mL), and high endonuclease concentrations (~100ng/mL). The % CV is the standard deviation divided by the mean and multiplied by 100.

Control	Intra assay CV	Inter assay CV
0.5 ng/mL	9.4%	9.4%
5 ng/mL	6.6%	4.1%
25 ng/mL	4.8%	4.3%
100 ng/mL	7.2%	7.7%

Accuracy

The accuracy of the assay was determined by testing a low (~0.5 ng/mL), two medium (~5 ng/mL and ~25 ng/mL) and a high (~100 ng/mL) sample and determining the percent nominal for each sample. Three independent preparations of each sample were tested, and 20 replicates were measured for each sample. Percentage Nominal was calculated: (Actual result divided by the theoretical result) x 100.

Pool	% Nominal
0.5 ng/mL	103%
5 ng/mL	100%
25 ng/mL	99%
100 ng/mL	110%

Recovery/ Interference Studies

Various buffer matrices commonly used in purification of therapeutic proteins and viral vectors as well as in-process and final formulation drug substances were evaluated by adding known amounts of G963 standard preparation used to make the standards in this Reagent Set. 11 samples yielded acceptable recovery defined as

between 80-120% (data not shown). Very high concentrations of some products may interfere in the accurate measurement of impurities. 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. **G960** is designed to be used in conjunction with Gyros Protein Technologies Cat. **P0020667**

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.gyrosproteintechologies.com/gyrolab-immunoassay-solutions

For product information on Gyrolab Bioaffy 1000 HC Assay Toolbox:

www.gyrosproteintechologies.com/gyrolab-bioaffy-1000-hc-assay-toolbox

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