EndonucleaseGTP® Solution for Gyrolab®

For the detection of endonuclease impurities in recombinant vaccines and viral vector products

Product Information Sheet D0040619/A

- Automated workflows – reduced manual operations
- Broad dynamic range – over three logs
- Fast turnaround – 96 data points in 75 minutes
- High throughput – up to 960 data points in a working day

Introduction

Optimizing downstream purification of biotherapeutics to meet regulatory requirements includes reducing the level of impurities that can induce toxic or immunogenic reactions in patients. Impurity levels are therefore a critical quality attribute. The production of recombinant vaccines and viral vector products, for example, can involve the addition of endonucleases such as Benzonase® or Denarase® during or after host-cell lysis to degrade nucleic acids and disrupt macromolecular complexes.

Monitoring the removal of these endonuclease impurities can be streamlined with analytical tools that provide broad dynamic range, automation, and fast turn-around time. In addition, the expense of vector production that results in a small volume of highly valuable product means that analytical methods requiring only a small volume of sample are at a premium.

To meet this need, the EndonucleaseGTP® Solution for Gyrolab® has been developed for sensitive detection of residual endonuclease. The solution, consisting of *EndonucleaseGTP Assay Reagent Set for Gyrolab* developed by Cygnus Technologies (Cygnus catalog number G960), and *Gyrolab Bioaffy 1000 HC Assay Toolbox*, quickly delivers high quality endonuclease quantification from sample volumes of less than 10 µL from different stages of a bioprocess. The reagent set has been fully tested and validated by Cygnus Technologies to generate high quality results on Gyrolab systems. It is derived from the same antibodies and antigen used in the Cygnus Endonuclease GTP 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.

Gyrolab systems, kits/solutions and software promise to help deliver timely analytical support in biopharmaceutical- and vector development and manufacture and streamline the implementation of Quality-by-Design (QbD) principles in bioprocess development and long-term monitoring.

EndonucleaseGTP Solution for Gyrolab increases productivity in bioprocess development:

- Automation generates 96 data points within 75 minutes without manual intervention
- Broad dynamic range minimizes dilutions needed to be in range and reduces the need for re-analysis
- Short turnaround time and reduced manual intervention accelerate data-driven decision making and free up operator time for more important tasks

GYROS PROTEIN Technologies

The Gyrolab solution

EndonucleaseGTP Solution for Gyrolab, with assay reagent set developed by Cygnus Technologies quantifies endonuclease impurities in bioprocess samples. The sandwich immunoassay is run on the Gyrolab Bioaffy 1000 HC CD (Figure 1). The biotinylated anti-endonuclease antibody is automatically introduced into a microstructure in the Gyrolab Bioaffy CD and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing endonuclease are introduced into the microstructures and captured by the immobilized anti-endonuclease antibody. Bound endonuclease is then detected using an anti-endonuclease antibody labeled with Alexa Fluor®647. Results are evaluated using Gyrolab Evaluator or exported to a LIMS. All Gyrolab software programs are designed for 21 CFR Part 11 compliance, ensuring that assays can be developed and transferred in regulated environments.

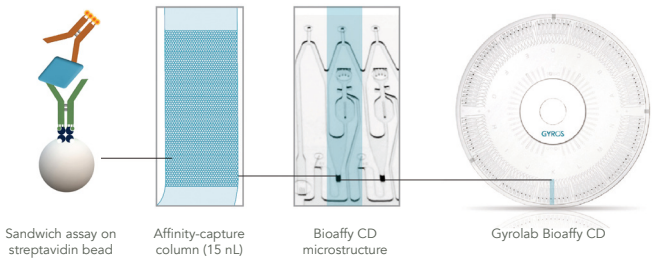

Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy 1000 HC CD

Assay performance

Broad dynamic range

The EndonucleaseGTP Assay Reagent Set for Gyrolab (G960), when used with the Gyrolab Bioaffy 1000 HC Assay Toolbox, demonstrates a broad, three-log working range (Table 1) that minimizes the number of dilutions needed to analyze bioprocess samples.

Table 1. Assay working range

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
~ 0.01	~ 0.05	200

LOD is determined as the concentration where the response equals two standard deviations above the average blank response. LLOQ and ULOQ are defined as the lowest and highest concentration respectively for which the CV is typically <20%.

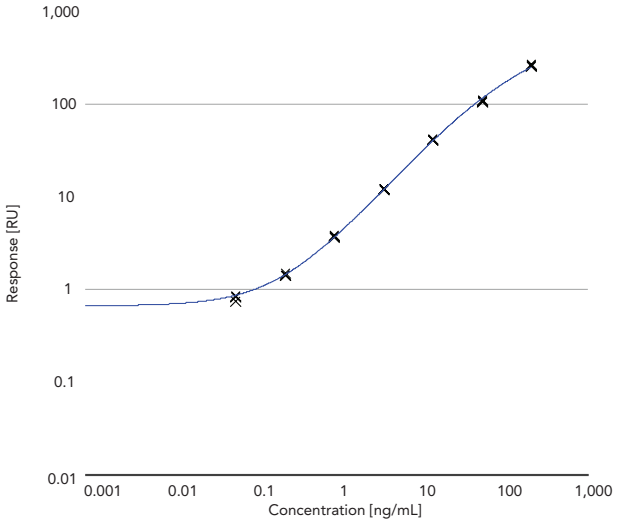

Figure 2. Typical standard curve data from an assay run

Precision

Intra- and inter-assay precision were determined at low (~0.5 ng/mL), medium (~5 ng/mL and 25 ng/mL), and high endonuclease concentrations (~100 ng/mL) (Table 2).

Table 2. Intra- and inter-assay precision

Concentration (ng/mL)	Intra-assay CV (%) n=20	Inter-assay CV (%) n=10
0.5	9.4	9.4
5	6.6	4.1
25	4.8	4.3
100	7.2	7.7

Accuracy

The accuracy of the assay was assessed by testing samples with low (0.5 ng/mL), medium (5 ng/mL and 25 ng/mL) and high (100 ng/mL) endonuclease concentrations. Three independent preparations of each sample were tested, and 20 measurements were performed for each sample (Table 3).

Table 3. Accuracy of the assay

Expected concentration (ng/mL)	% Nominal*
0.5	103
5	100
25	99
100	110

*% Nominal = (measured conc./expected conc.) x 100

Recovery/Interference Studies

Various buffer matrices commonly used in the purification of therapeutic proteins and monoclonal antibodies as well as in-process and final formulation drug substances were evaluated by Cygnus Technologies by adding known amounts of G963 standard preparation used to make the standards in the Cygnus Reagent Set. A total of 11 samples yielded acceptable recovery, defined as being in the range 80–120% (data not shown).

An industry partner tested five (5) different matrices representing different stages of bioprocessing by adding 10 ng/mL of G963 standard preparation. Spike recoveries were performed on each of the matrices to assess the matrix effect on the accuracy of the measurement. Spiked samples demonstrate dilutional linearity from 1 in 2 to 1 in 32 across all the matrices (Table 4).

Table 4. Percent (%) recovery for five different matrices spiked with 10 ng/mL of EndonucleaseGTP standard.

Matrix (spiked to 10 ng/mL)	Dilution	Predicted Endonuclease Conc. (ng/mL)		% Recovery
		Mean	% CV	
1	2	9.7	0.7	97.1
	4	10.5	3.3	105.4
	8	10.1	5.6	100.7
	16	9.7	9.6	97.0
	32	10.2	14.5	102.4
2	2	8.8	5.4	87.8
	4	8.9	2.6	89.0
	8	8.2	9.4	82.2
	16	8.1	3.8	81.1
	32	8.1	3.2	80.5
3	2	8.3	4.4	82.5
	4	7.4	3.2	74.5
	8	7.1	3.1	70.8
	16	7.2	5.0	71.7
	32	7.2	2.4	71.5
4	2	9.2	4.9	92.3
	4	9.3	0.7	93.2
	8	9.1	26.8	91.4
	16	8.5	23.0	84.9
	32	7.4	7.1	74.0
5	2	6.2	0.6	62.2
	4	6.6	0.1	65.5
	8	7.0	5.2	69.9
	16	6.0	7.0	60.0
	32	6.3	0.5	63.3

Very high concentrations of some products may interfere in the accurate measurement of contaminants. Each user should validate that their sample matrices yield accurate recovery.

Due to the fact that manufacturers of endonuclease enzymes report their respective enzyme specific activity in units/mg and the fact that specific activity reflects active enzymatic activity per mg protein while not accounting for the inactive enzyme mass, you can expect up to 3X recovery value for a given standard provided in the EndonucleaseGTP Assay Reagent Set for Gyrolab (Cygnus Cat. #G960), as compared, for example. to Merck KgaA Benzonase ELISA Kit II. Cygnus recommends preparing a positive control using your specific endonuclease to better assess the expected differences.

Specificity/Cross-Reactivity

Cross reactivity to non-endonuclease components has not been extensively investigated with EndonucleaseGTP Solution for Gyrolab.

Initial data generated by an industry partner show good specificity when assay was challenged using:

- Empty AAV5 capsids (generated at customer site).
- Ligand standard from a common residual ligand assay.
- Host cell protein standard from a commercial ELISA kit.

Response from bottom standard (0.039 ng/mL) was 0.577 R. All non-specific samples were less that the lowest standard (Table 5).

Table 5. Mean responses and %CV for three different test samples.

Test sample	Response (RU)		
	Mean	Std Dev	% CV
Empty AAV5 capsids (1E11 VP/mL)	0.394	0.028	7.1
Capture ligand standard (20 ng/mL)	0.428	0.015	3.6
HCP (20 ng/mL)	0.439	0.057	12.9

Customers are advised to evaluate components in their own samples for positive interferences such as cross-reactivity and non-specific binding.

Ordering Information

Product Number	Product name	Description	Supplier
G960	EndonucleaseGTP Assay Reagent Set for Gyrolab	Contains anti-endonuclease capture and detection reagents and endonuclease standard. Quantities enough to generate 96 data points (1 CD).	Order from Cygnus Technologies
P0020667	Gyrolab Bioaffy 1000 HC Assay Toolbox	Contains all reagents and consumables needed to generate 96 data points (1 CD).	Order from Gyros Protein Technologies
P0020668	Gyrolab Bioaffy 1000 HC Assay Toolbox CD50	Contains all reagents and consumables needed to generate 4800 data points (50 CD's).	Order from Gyros Protein Technologies
P0020670	Gyrolab HCP Sample Dilution Buffer 25 mL	Extra sample dilution buffer for Gyrolab Bioaffy 1000 HC Assay Toolbox.	Order from Gyros Protein Technologies

Content

Gyrolab Bioaffy 1000 HC Assay Toolbox

Each toolbox contains buffers and consumables for one (1) or fifty (50) CDs, for generation of 96 or 4800 data points, respectively.

Storage conditions

Gyrolab Bioaffy 1000 HC Assay Toolbox

Refrigerate at +4°C to +8°C. Do not freeze.

Shelf life (unopened package): see product label.

Related products

Scan the QR-code to learn more about our other ready-to-use kits and solutions used for bioprocess analytics:

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