Gyrolab® p24 Kit

For lentivirus titer determination

Product Information Sheet D0034387/C

Benefits of performing lentivirus titer determinations with Gyrolab p24 Kit:

- **Accelerate lentivirus bioprocess workflows and reduce time to market**
  - Fast results for data driven decisions – 96 data points in 80 minutes
  - Automated analyses – less manual operations
  - High throughput – up to 960 data points in one working day
- **Generate high data quality from small sample volumes**
  - 10 x less sample required compared to ELISA
  - Broad dynamic range and low matrix interference reduces the need for dilutions

Introduction

Lentiviral vectors based on HIV-1 and EIA viruses are commonly used as vehicles to transfer therapeutic genes. Improvements in vector design that increase biosafety and transgene expression have led to the approval of lentiviral vectors for use in clinical studies as well as the commercial approval of new therapies. Lentiviral vectors can be used *ex vivo*, for example in the preparation of CAR T cells for the treatment of acute lymphoblastic leukemia, and in direct *in vivo* use, for example in the treatment of Parkinson's disease and rare retinal diseases.

Manufacturing lentiviral vectors involves cell culture, often based on HEK 293 cell lines. The characterization and quality control of lentivirus manufacturing processes is complex and includes determining virus titer and impurity levels. Virus titer can be determined by measuring levels of viral proteins such as the p24 capsid protein of HIV-1.

Gyrolab Systems have become proven and established analytical tools for bioprocess development and manufacturing. Features that increase the productivity in bioprocess workflows include automated analysis, broad analytical range, high quality results, and software designed for 21 CFR part 11 compliance.

To meet the need to determine lentivirus titers, Gyros Protein Technologies has developed Gyrolab p24 Kit. Gyrolab p24 Kit is ready to use and works over a broad analytical range. Use of Gyrolab Systems in combination with ready to use kits promises to accelerate bioprocesses involving viral vector production and reduce the time to market.

GYROS PROTEIN Technologies

The assay

Gyrolab p24 Kit has been developed to quantify the HIV-1 capsid protein p24 in cell culture samples containing lentiviral vectors. The kit is based on a sandwich assay that uses a biotinylated capture molecule and a detection molecule labeled with Alexa Fluor® 647.

The kit contains ready-to-use capture- and detection reagents that are pre-labeled with biotin and Alexa Fluor® 647, respectively. Standards are pre-diluted, which minimizes pipetting errors and maximizes analysis consistency and quality of results. The biotinylated anti-p24 antibody is automatically introduced into a microstructure in Gyrolab Bioaffy 1000 CD and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing p24 are introduced into the microstructures and captured by the immobilized anti-p24 antibody. Bound p24 is then detected using the fluorophore-labeled anti-p24 detection antibody (Figure 1). 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.

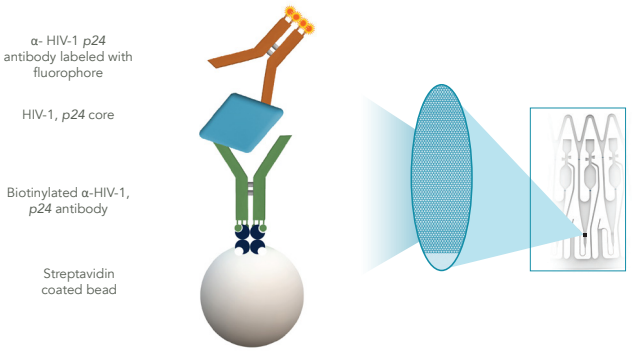

Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy 1000 CD.

Assay performance

Gyrolab p24 Kit has a broad, three-log working range (Table 1) that minimizes the number of dilutions needed to analyze bioprocess samples with concentrations ranging from µg/mL down to ng/mL. LLOQ and ULOQ have been established in six runs as the concentrations where Total Error (%CV + absolute %RE) < 30%, (Table 2). LOD was determined as the concentration where the response equaled two standard deviations above the average blank response.

Table 1. Assay working range.

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
<0.05	~0.2	~1 000

Table 2. Accuracy and precision data for five QC samples for determination of working range.

Sample	Exp conc (ng/mL)	Average measured conc (ng/mL)	Intra-run CV (%)	Inter-run CV (%)	TE range (%)
QC1	1000	1054	4.2	6.0	4.4-21.8
QC2	800	889	4.6	4.1	13.1-18.7
QC3	10	10.0	2.0	3.4	2.1-6.9
QC4	0.3	0.31	2.3	2.3	2.7-6.3
QC5	0.2	0.2	2.9	4.1	2.5-9.4

Table 3. Intra- and inter-run precision data for the standard curve over the assay working range.

	Expected conc (ng/mL)	Average measured conc (ng/mL)	Intra-run CV (%)	Inter-run CV (%)
Blank	0			
Standard 1	1250	1250	3.6	3.1
Standard 2	250	251	2.3	1.9
Standard 3	50	50	2.8	2.7
Standard 4	10	10	2.9	2.8
Standard 5	2	2	1.7	2.1
Standard 6	0.4	0.4	2.0	1.8
Standard 7	0.08	0.08	5.0	5.3

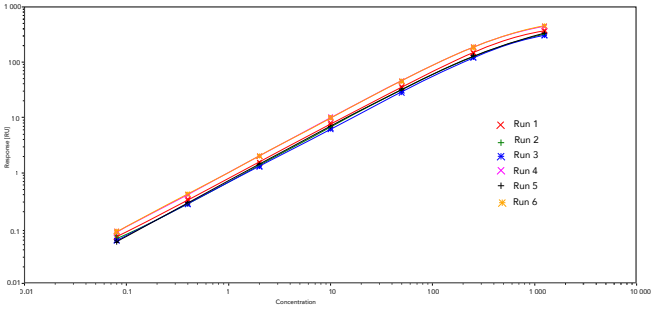

Figure 2. Overlay plot from standard curves from six runs on four instruments.

Data for standard curves and QC samples over the working range run in duplicate in six runs on four instruments by three operators are shown in Figure 2, Table 2 and Table 3.

p24 determination in virus samples

p24 is a component of the lentivirus particle capsid. In cell culture supernatants p24 can also be present free in solution. p24 is commonly used to determine titer in lentiviral production. To release the virus-associated p24 the virus preparation must be treated with a suitable detergent, such as Triton™ X-100, to lyse the viral membrane (Figure 3a and 3b).

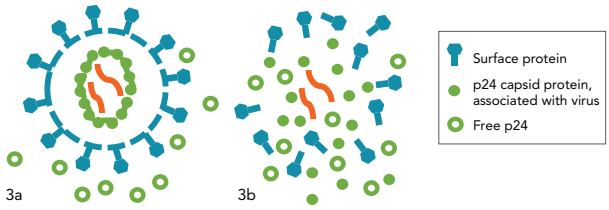

Figure 3a. Untreated virus particles.
Figure 3b. Treated with Triton X-100.

The ratio between free- and virus associated p24 may vary between samples from different steps in the purification process or between different virus processes. Table 4 shows the average p24 concentrations in five virus preparation samples, treated or untreated with Triton X-100, run in duplicate. The results demonstrate that Gyrolab p24 Kit can determine the quantity of p24 in virus preparation samples that are un-treated or treated with Triton X-100.

Table 4. Comparison of concentrations determined in five virus preparation samples, un-treated or treated with Triton X-100..

Identity	Average conc + Triton (ng/mL)	Average conc No Triton (ng/mL)	Ratio + Triton/No Triton
Sample 1	9651	498	19.4
Sample 2	411	112	3.7
Sample 3	782	292	2.7
Sample 4	3582	431	8.3
Sample 5	40094	829	48.4

Dilutional linearity

Linearity of the assay was established with two samples diluted in four steps and demonstrated that the sample matrix had little effect on assay performance. Table 5.

Table 5. Dilutional linearity.

Identity	Dilution Factor	Back-calculated conc (ng/mL)	CV (%)	Average Back calc. conc. (ng/mL)
Sample 1 No Triton	2	107	1.6	113
	4	112	3.4	
	8	119	6.4	
	16	115	3.6	
Sample 1 + Triton	2	418	1.2	428
	4	450	3.6	
	8	445	3.8	
	16	397	7.9	
Sample 2 No Triton	2	421	3.3	427
	4	429	0.8	
	8	428	2.4	
	16	429	3	
Sample 2 + Triton	2	3962	7.3	3847
	4	4335	1.1	
	8	3620	7.5	
	16	3471	8	

Spike recovery

Table 6 shows the excellent spike recovery of viral preparation samples that have been spiked with standard, demonstrating the accuracy of the kit.

Table 6. Results from spike recovery experiments.

Identity	Spiked		Unspiked			
	CV Conc (%)	Back calc conc (ng/mL)	CV Conc (%)	Back calc conc (ng/mL)	Spike conc. (ng/mL)	Spike recovery (%)
Sample 1 No Triton	4.4	851	2	522	330	100
Sample 1 + Triton	2.9	12646	4.5	9147	3300	106
Sample 2 No Triton	0.3	216	1.7	110	110	97
Sample 2 + Triton	2.7	718	4.3	394	330	98
Sample 3 No Triton	1.7	381	1.1	292	110	81
Sample 3 + Triton	5.3	1117	5.3	784	330	101

Abbreviations used: HIV, human immunodeficiency virus; EIA, equine infectious anemia; CAR, chimeric antigen receptor; HEK, human embryonic kidney; LIMS, laboratory information management system; LLOQ, lower limit of quantitation; ULOQ, upper limit of quantitation; LOD, limit of detection; CV, coefficient of variation; TE, total error.

Ordering Information

Product Number	Product name	Description
P0020659	Gyrolab p24 Kit	Reagents and consumables to generate 96 data points.
P0020665	Gyrolab p24 CD50 Kit	Reagents and consumables to generate 4800 data points (50x96 datapoints).
P0020664	Gyrolab p24 Standard	1250 ng/mL, 400 µL. For spiking experiments.
P0020674	Gyrolab p24 Sample Dilution Buffer	Extra sample dilution buffer, 25 mL.

Gyrolab p24 Kit Contents

Each kit contains reagents and consumables for one (1) or fifty (50) CDs, for generation of 96 or 4800 datapoints respectively:

- Gyrolab p24 Kit Reagents (for contents, see below)
- Gyrolab Bioaffy 1000 CD
- Gyrolab Wash buffer pH 11
- 96-well plate
- Microplate Foil

Gyrolab p24 Kit Reagents

- Capture Reagent: Biotinylated anti-HIV-1 p24, ready-to-use solution
- Detection Reagent: Fluorophore-labeled anti-HIV-1 p24, ready-to-use solution
- Recombinant p24 Standard: 8-well strip containing the ready-to-use standard curve samples
- Wash Buffer
- Sample Dilution Buffer

Storage conditions

Gyrolab Bioaffy 1000 CD

Refrigerate at +4 °C to +8°C, unopened package.

Shelf life (unopened package): Minimum 12 months after delivery.

Gyrolab p24 Kit Reagents

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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