SARTORIUS

Product Datasheet

Virotag® DY ENV Reagent Kit

Reagent for the Virus Counter® Platform

Product Information

Virotag® DY ENV reagents are ready-to-use quantification kits for use on the Virus Counter® Platform. The Virus Counter® 3100 allows customers rapid, direct and precise quantification of viruses with results in less than one hour. Near real-time insights into virus titers allow optimization of development and manufacturing processes and improving virus yields. Virotag® DY ENV reagent kits are optimized for use with the Virus Counter® 3100 platform to detect and precisely enumerate enveloped viruses. These kits contain all necessary reagents and consumables for operating the Virus Counter® 3100 instrument. The Virotag® DY reagent included in these kits is a two-component dye reagent that stains both protein and nucleic acid, allowing for the detection of total virus particles on the Virus Counter instrument.

The Virotag® DY ENV reagent is optimized for enveloped viral particles that contain a viral genome[1]. Due to the general nature of the stain, no prior knowledge of genetic sequence or envelope proteins is necessary for a successful assay.

[1] Sample matrix components or crude sample matrix may interfere with particle detection. Individual sample compatibility must be verified by the customer.

Benefits

- Ready-to-use, universal quantification solution for enveloped viruses using the Virus Counter® 3100 Platform
- Shortened time-to-result for critical experiments that require virus particle quantification
- Ease-of-use with simple, no-wash assays and software-guided sample analysis
- No prior knowledge of genomic sequences or epitopes required

Application Examples

- The Virus Counter® 3100 platform is purpose built to rapidly and precisely quantify total virus particles in process development and manufacturing.
- Accelerate development times with real-time virus titer insights into up- and downstream processes for vaccines and viral therapies.
- Improve product yield by optimizing development and manufacturing processes.
- Increase product safety and quality by in-depth sample characterization.

Product Description

Virotag® DY ENV enables detection of enveloped viruses by staining both nucleic acid and proteins with fluorogenic dyes. The nucleic dye is an intercalating agent that binds both single- and double-stranded DNA and RNA molecules, allowing for the staining of any viral genome of sufficient size. The protein dye binds the hydrophobic domains of proteins, such as the transmembrane regions that transit the viral envelope.

During analysis, stained particles pass through the flow cell of the Virus Counter® instrument where they are excited by a laser. The dyes emit at two distinct wavelengths as they pass through the light, and these signals are detected using separate photomultiplier tubes (PMTs). Only instances where signals from both dyes are detected by both PMTs simultaneously are quantified as virus particles. This approach allows for the rapid, direct, and specific quantification of viral particles in a sample.

Sample staining is a simple, no-wash assay that can be completed in 30 minutes. The procedure is as follows:

1 Dilute virus sample into Sample Dilution Buffer.
2 Add 5 µL Virotag® DY Reagent to a clean Sample Vial.
3 Add 195 µL diluted virus sample to the Sample Vial.
4 Mix thoroughly by pipette.
5 Close Sample Vial with Cap.
6 Incubate the sample(s) in the dark at room temperature for at least 30 minutes.
7 Analyze samples with the Virus Counter® instrument using the Virotag® DY stain setting. Results will be reported as virus particles per milliliter (vp/mL).

Virus samples stained with Virotag® DY reagent may be stable for up to 24 hours under proper storage conditions.[2] For best results, measure samples within four hours of staining if leaving the samples at room temperature. If samples must be left longer, place at 4 °C and keep protected from light.

[2] Customers are recommended to determine the stability of their samples for optimal results.

Technical Data

The flexibility of the Virotag® DY ENV reagent kit allows for the rapid quantification of viruses without the need for target-specific detection reagents (such as antibodies for ELISA or sequence-specific primers for qPCR). The Virotag® DY ENV reagent kit allows for the rapid quantification of enveloped viruses, such as Influenza virus, Lentivirus, and Baculovirus. Samples are stained using a single-step, no-wash staining protocol using Virotag® DY reagent, Sample Dilution Buffer, and the sample of interest. After a 30-minute incubation period, stained samples are measured on the Virus Counter® instrument to detect viral particles and determine sample titers.

To precisely assess the titer of a viral sample, the approximate particle concentration of the virus sample must be determined with an initial screening assay to allow for the selection of an optimal dilution factor for replicate measurement and titer determination. This screening assay is accomplished by preparing a dilution series of the sample of interest to determine the optimal dilution factor. The optimal dilution factor will yield results between 1×10^7 and 5×10^7 vp/mL. The Virus Counter® instrument has a dynamic range of 5×10^5 vp/mL to 1×10^9 vp/mL. Below 5×10^5 vp/mL (also known as the Instrument Quantification Limit, or IQL) counting variability can decrease the precision of results. Above 1×10^9 viral particles may begin to crowd as they transit the detector, an occurrence known as coincidence. Coincidence results in inaccurately low counts and must be avoided during titer determination.

For all assays, including the screening assay, negative controls should be prepared and analyzed along with the sample(s) of interest. These negative controls approximate the matrix the virus sample is kept in (examples include formulation buffer or conditioned media) diluted to the same

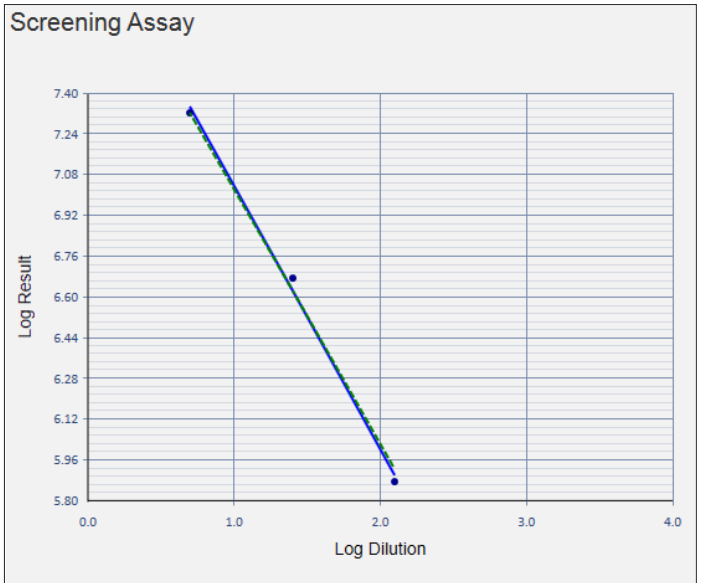
factor as the sample. If sample matrix is unavailable, Sample Dilution Buffer stained with Virotag® DY reagent is a suitable negative control.

Once the range of optimal dilution factors is found in the screening assay, the sample titer may be determined by measuring replicates at a dilution factor within this range. The following section describes this procedure in depth.

Screening Assay

A dilution series of Fort Morgan Virus was prepared from a crude sample and stained using the procedure previously described. Fort Morgan Virus dilutions (1 : 125, 1 : 25, and 1 : 5) and a Sample Dilution Buffer blank were analyzed using the Virus Counter platform. Results were then imported to the Screening tab found in the Virus Counter® application to determine the optimal dilution range for titer determination.

The Screening function of the Virus Counter® application takes the dilution series data and determines the linear response of the sample (Figure 1), allowing for the calculation of dilution factors that will provide results within the middle of the dynamic range (1×10^7 to 5×10^7) (Table1).



Target Count	Log Count	Slope	Intercept	Log Factor	Dilution Factor
5.0E+06	6.7	-1.04	8.0705	1.32	21
1.0E+07	7.0	-1.04	8.0705	1.03	11
5.0E+07	7.7	-1.04	8.0705	0.36	2
1.0E+08	8.0	-1.04	8.0705	0.07	1

Figure 1: Screening Assay results for Fort Morgan virus. Virus Counter® results were corrected for background. Dilution factors and results were then log-transformed and the results fit with a line. The acceptable slope values are -0.7 to -1.3. Acceptable R^2 (a goodness-of-fit parameter) is ≥0.9. The slope and intercept values are used to predict a dilution factor that will yield counts in the range where the Virus Counter® achieves greatest precision.

Table 1: Suggested dilution factors for a range of target results. Highlighted in green is the range that will yield the greatest precision. In red are values that are not advisable due to the potential to clog the instrument.

Target Count	Log Count	Slope	Intercept	Log Factor	Dilution Factor
5.0E+06	6.7	-1.04	8.0705	1.32	21
1.0E+07	7.0	-1.04	8.0705	1.03	11
5.0E+07	7.7	-1.04	8.0705	0.36	2
1.0E+08	8.0	-1.04	8.0705	0.07	1

Based on recommended dilution factors, a final dilution of 1 : 10 was chosen for determining the titer of the sample.

Titer Determination

Using the target dilution factor determined by the Screening function, five Fort Morgan Virus replicate samples and three blank samples were stained as previously described. Data was imported into the "Titer" tab of the Virus Counter® software and the final titer for the Fort Morgan sample was determined using the three blank replicates for data correction (Table 2).

Table 2: Results of titer determination for Fort Morgan virus. The average titer, standard deviation, and percent C.V. are calculated from background-corrected sample data.

Effective Dilution Factor	10
Average Titer	1.3E+08
Standard Deviation	1.6E+07
%C.V.	12.25%

In the study presented herein, representative members of different virus families known to harbor viruses with the potential to emerge as novel pathogens were used to demonstrate the broad utility of Virotag® DY ENV for the quantification of pathogens. In situations where a novel and athogenic virus jumps into a naïve population, such as the COVID-19 pandemic of 2020 caused by a novel Coronavirus (SARS-CoV-2), it is critical to be able to rapidly begin research on effective vaccines and treatment options. Virotag® DY ENV and the Virus Counter® platform effectively support these efforts by allowing for the rapid quantification of enveloped virus samples without the need for virus-specific reagents.

Viruses selected for this study were Fort Morgan Virus (Alphaviridae), Tacaribe virus (Bunyaviridae), Coronavirus 229E (Coronaviridae), Zika virus (Flaviviridae), and Influenza A/Michigan/45/2015 (Orthomyxoviridae). Data was collected for each virus in the manner described in the previous section. Figure 2 shows the linear response of each virus in dilution series across the dynamic range of the Virus Counter®. Figure 3 compares the Virus Counter® 3100 titer data with $TCID_{50}$ data from the vendors of the samples. This data is tabulated in Table 3.

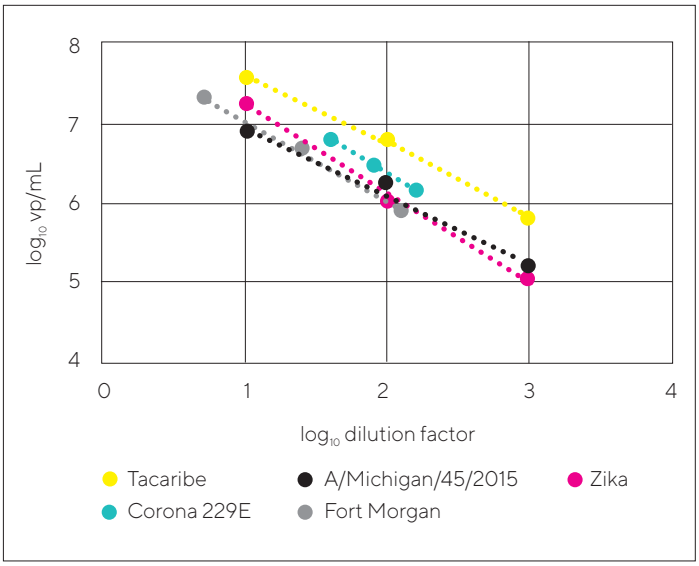


Figure 2: Linear responses of various species of enveloped viruses stained with the Virotag® DY ENV kit.

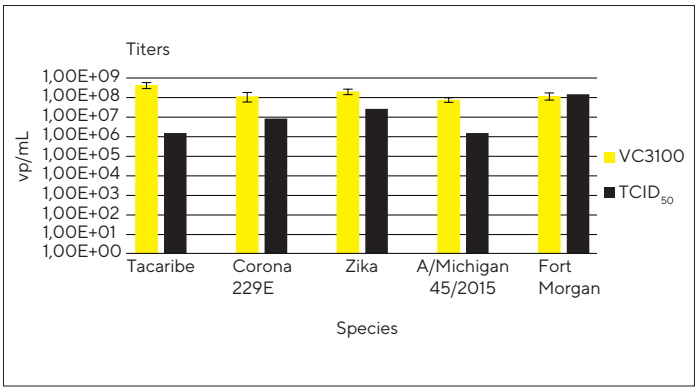


Figure 3: Titer data from the Virus Counter® 3100 (VC3100) compared to $TCID_{50}$ data supplied by the virus vendors. This data highlights the often wide disparity between infectious and non-infectious particles within a viral preparation.

Table 3: Titer data. Range reported for Virus Counter® 3100 titer values is the 95% confidence interval for five replicate points. The ratios of total particles to infectious particles are rounded to the nearest integer. Values in parentheses cover the 95 percent confidence interval.

Family	Species	Strain	Titer (vp/ml)	% c.v.	$TCID_{50}$ (/mL)	Total particle to infectious particle ratio
Arenavirus	Tacaribe	TRVL-11573	$4.70 \times 10^8 \pm 1.31 \times 10^8$	10.0	8.90×10^6	297(214-380)
Coronavirus	Human coronavirus	229E	$1.24 \times 10^8 \pm 5.60 \times 10^7$	16.3	1.58×10^6	14(8-20)
Flavivirus	Zika	PRVABC59	$2.20 \times 10^8 \pm 5.31 \times 10^7$	8.70	2.80×10^7	8(6-10)
Orthomyxovirus	Influenza	A/Michigan/45/2015	$8.19 \times 10^7 \pm 1.68 \times 107$	7.39	1.60×10^6	51(41-62)
Togavirus	Fort Morgan	CM4-146	$1.29 \times 10^8 \pm 4.49 \times 10^7$	12.4	1.58×10^8	1(1-1)

Ordering Information

VIR-92341	Virus Counter® 3100 Instrument	
VIR-92416	Virotag® DY ENV Kit 200 Assays	Universal quantification of enveloped viruses, 200 assays per kit
VIR-92417	Virotag® DY ENV Kit 100 Assays	Universal quantification of enveloped viruses, 100 assays per kit
VIR-92418	Virotag® DY ENV Kit 50 Assays	Universal quantification of enveloped viruses, 50 assays per kit

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