ViroCheck NanoParticle Reference Kit

Catalog Number V10425

Pub. No. MAN0026327 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The ViroCheck NanoParticle Reference Kit (Cat. No. V10425) contains 100 nm, 200 nm, and 500 nm particles with assigned intensity values referred to as Equivalent Reference Fluorophores (ERF). The NIST-assigned standards are for individual channels within a fluorophore's selected band-pass filter resulting in accurate, quantitative, and comparable flow cytometry fluorescence intensity measurements.

The ViroCheck NanoParticle Reference Kit features:

- NIST-assigned ERF values for 15 common filter set channels (ERF values for filter sets not listed can be provided upon request)
- Enables fluorescence intensity measurements for instrument accuracy and sensitivity
- Facilitates instrument to instrument data comparison
- Enables submicron (i.e. Virus and EV) sample concentration measurements

Contents and storage

Component	Excitation laser and Emission filter set				Measured concentration (beads/mL)	Storage
	UV (375 nm)	Blue (488 nm)	Yellow (561 nm)	Red (633 nm)		
100 nm FITC-like beads	—	530/30 574/26 610/20	—	—	N/A	2°C to 8°C
100 nm PE-like beads	—	574/26	585/16 620/15	—	N/A	
100 nm APC-like beads	—	—	—	660/20 670/14 720/30 780/60	N/A	
200 nm Multicolor beads	405/30 450/45 525/40	530/30 574/26 610/20	585/16 610/20 675/30 695/40	660/20 670/14 720/30 780/60	Yes[1]	
500 nm Multicolor beads	405/30 450/45 525/40	530/30 574/26 610/20	585/16 610/20 675/30 695/40	660/20 670/14 720/30 780/60	Yes[1]	

[1] See component label for particle concentration.

Experimental protocols

Protocol to determine ERF value of the sample

Note: Before you run your experiment, wash your flow cytometer thoroughly. We recommend using 0.02 to 0.05-micron filtered buffers.

1. Select the ViroCheck reference nanoparticles that are needed for your experiment. Pipette the nanoparticles up and down to resuspend.
2. Add 20 µL of ViroCheck reference nanoparticle suspension per 1 mL of sample buffer. Vortex briefly to mix.
3. Run the reference nanoparticles on a flow cytometer. Adjust instrument voltage settings to visualize the nanoparticles on a fluoroscence intensity vs. side scatter dot plot.

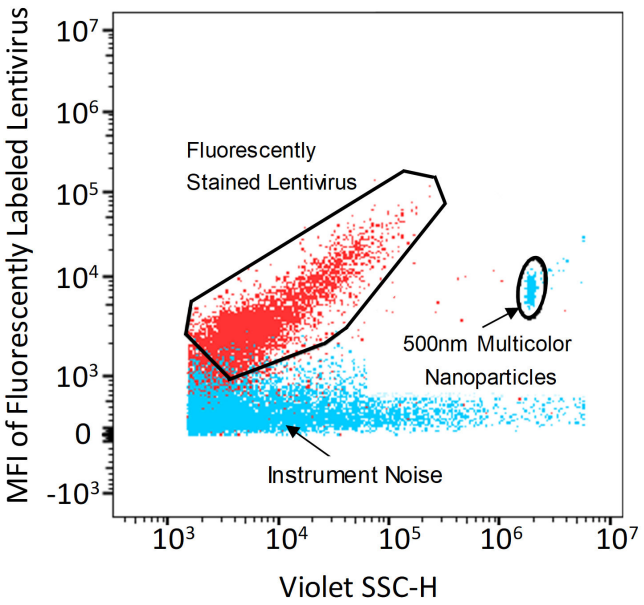

Figure 1 Fluorescence intensity versus side scatter (SSC) dot plot for fluorescently stained lentivirus particles and 500 nm Multicolor nanoparticles

Note: When running multiplex experiments that require compensation, begin by adjusting voltage settings using your single-color controls. ViroCheck NanoParticle Reference Kit will be run using these settings.

4. Run the stained sample using the same instrument settings.

Protocol to determine the concentration of the sample

Note: Before you run your experiment, wash your flow cytometer thoroughly. We recommend using 0.02 to 0.05-micron filtered buffers.

1. Process your submicron samples as needed (i.e., fluorescent staining).
2. Select the ViroCheck reference nanoparticles that are needed for your experiment. Pipette the nanoparticles up and down to resuspend.

   Note: We recommend 200 nm or 500 nm Multicolor beads for best accuracy.
3. Add 20 µL of ViroCheck reference nanoparticle suspension to each sample. Vortex briefly to mix.
4. Run the reference nanoparticles on a flow cytometer. Adjust instrument voltage settings to visualize the nanoparticles on a fluoroscence intensity vs. side scatter dot plot (Figure 1).
5. Run the stained sample using the same instrument settings.
6. Use normal gating strategies to identify the reference nanoparticle population and the sample population to be enumerated.
7. Using the count statistics from the sample and reference nanoparticle gates, determine the original sample concentration using the equations listed in “Calculations” on page 3.

Calculations

ERF calculation

1. For use in the sample ERF calculation, select the nanoparticle peak that most closely matches the MFI of your sample (i.e., nearest neighbor principle).

2. Use the following equation to calculate the ERF value of your sample:

$$\text{ERF of Sample} = \text{ERF of Reference Particle} \times \frac{\text{MFI of Reference Particle}}{\text{MFI of Sample}}$$

Example calculation

The ERF value for a fluoroscently stained sample measured on a flow cytometer using a 488-nm excitation laser and a 530/30 nm emission filter set is calculated as follows:

1. ERF value of FITC-like 100-nm bead in the 530/30 filter set is 2,380.
2. MFI value of FITC-like 100-nm bead in the 530/30 filter set is 25,835.
3. MFI value of fluoroscently stained sample in the 530/30 filter set is 13,181.
4. $MFI_{sample}/MFI_{FITC\text{-}like\ bead}$ ratio is 0.5102.
5. ERF value of sample is 2,380 × 0.5102 = 1,214 (ERF units).

Sample concentration calculation (Absolute count)

When determining the absolute count, which is defined as the concentration of particles in the original sample added to the flow cytometry tube, only the ratio of the measured volumes of the sample particles and the selected reference nanoparticles from the ViroCheck NanoParticle Reference Kit are included in the calculation. Additional volumes of staining buffer and other buffers do not need to be included when calculating absolute counts.

When using the protocol to determine the sample concentration, use the following equation to calculate the absolute count:

$$\text{Absolute Count} \left(\frac{\text{particles}}{\mu\text{L}}\right) = \frac{\text{Sample Count} \times \text{Ref. Particle Vol.}}{\text{Ref. Particle Count} \times \text{Sample Vol.}} \times \text{Ref. Particle Conc.}^*$$

* Reference nanoparticle concentration (particles / µL) from the product label.

Example calculation

1 mL of virus with an unknown concentration was stained. Afterwards, 20 µL of the 200 nm Multicolor reference beads were added at a label concentration of 1.00×10^5 particles/20 µL. On the flow cytometer, the number of virus events and the 200 nm Multicolor bead events were determined to be 1,700 and 1,030, respectively.

$$\frac{1{,}700\ \text{Virus Particles} \times 20\ \mu\text{L}}{1{,}030\ \text{Ref. Particles} \times 1{,}000\ \mu\text{L}} \times \frac{100{,}000\ \text{Ref. Particles}}{20\ \mu\text{L}} = 165\ \frac{\text{Virus Particles}}{\mu\text{L}}$$

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	15 February 2022	New document.