AccuCheck ERF Reference Particles

Catalog Number A55950

Pub. No. MAN0019837 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 Invitrogen™ AccuCheck ERF Reference Particles (Cat. No. A55950) are ~3 µm particles with assigned intensity values referred to as Equivalent Reference Fluorophores (ERF). The NIST-traceable standards are for individual channels within a fluorophore's selected band-pass filter resulting in accurate, quantitative and comparable flow cytometry fluorescence intensity measurements.

Experimental protocol

1. Briefly vortex AccuCheck ERF Reference Particles to resuspend. Add one drop of AccuCheck ERF Reference Particles to a flow cytometry tube.
2. Add 1 mL of sample buffer. Vortex briefly to mix.
3. Run particles on a flow cytometer. Adjust instrument voltage settings to allow all 3 intensity peaks to appear on a histogram plot of particle count vs. MFI. (Figure 1).

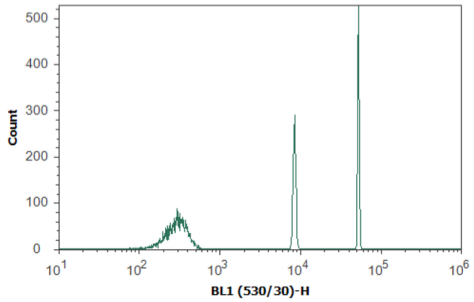

Figure 1 Multiple Fluorescence Intensities of AccuCheck ERF Reference Particles

Each of the 26 emission filters referenced by the AccuCheck ERF Reference Particles are reported at 3 levels of intensity.

Note: When running multiplex experiments requiring compensation, begin by adjusting voltage settings using your single color controls. AccuCheck ERF Reference Particles will be run using these settings.

4. Run stained sample using the same instrument settings.

Calculations

Method #1 (preferred method)

1. Create a standard curve by plotting ERF values for each AccuCheck ERF Reference Paricle (see provided insert for lot specific ERF values) vs MFI of each peak (from "Experimental protocol", step 4) as shown in Figure 2.

   Note: If filter set does not match those reported on the insert, please call tech support to obtain adjusted values.

2. Determine the ERF value of your sample by locating the ERF value that corresponds with the MFI value that intersects on your standard curve.

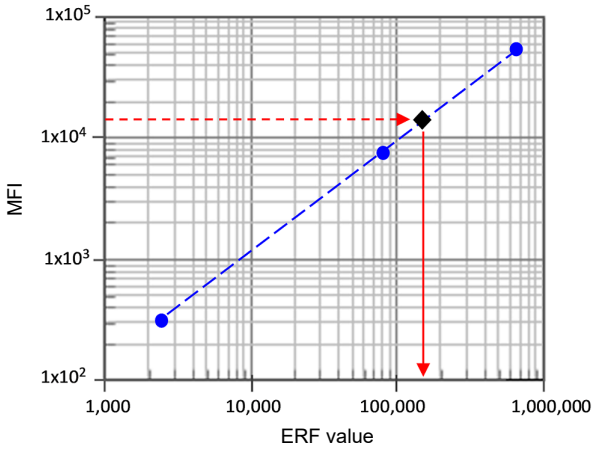

Figure 2 Fluorescence intensity determination of a sample – Graphing method

MFI values for the FITC filter set were collected by running AccuCheck ERF Reference beads on an Attune™ NxT Flow Cytometer. A plot of FITC MFI vs lot specific ERF values was used to generate a standard curve. Locate the corresponding ERF value of your sample (black data point) by determining

where the sample MFI maps to on the standard curve (see red arrows).

	ERF FITC (530/30)	MFI (530/30)
Low	2,380	315
Medium	80,300	8,725
High	617,000	54,209

Method #2

1. For use in the calculation, select the particle 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 the sample:

$$\textit{Intensity peak ERF value} \times \frac{\textit{MFI sample}}{\textit{MFI intensity peak}} = \textit{ERF value of sample}$$

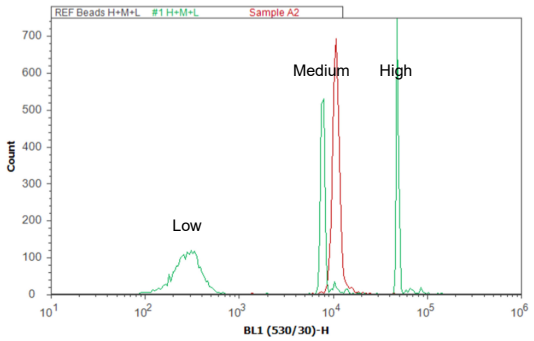

Figure 3 Fluorescence Intensity determination of a sample – Nearest neighbour method

When the signal of a sample (red histogram peak) is overlaid on an intensity plot of AccuCheck ERF Reference beads (low, medium, high histogram peaks), the ERF value for the particle peak that most closely matches the sample is selected to include in the calculation outlined in step 2.

	MFI (530/30)	ERF FITC (530/30)
Low	315	2,380
Medium	**8,725**	**80,300**
High	54,209	617,000
Sample	**13,181**	***121,310***

Example of calculating ERF value (FITC labeled cells) using the "nearest neighbor" reference calibration method, where the sample most closely matches the medium value.

1. ERF value of medium intensity beads is 80,300
2. MFI value of medium intensity beads is 8,725
3. MFI value of FITC labeled cells is 13,181
4. MFI_{sample}/MFI_{ERF} ratio is 1.5107
5. ERF value of sample is 80,300 × 1.5107 = 121,310 (ERF units, FITC)

Limited product warranty

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Life Technologies Corporation | 29851 Willow Creek | Eugene, OR 97402
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