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FCMPASS - Fluorescence calibration

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1 Works for me dx.doi.org/10.17504/protocols.io.bhvyj67w

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ABSTRACT

This protocol outlines the steps required to input fluorescence calibration parameters using the FCMPASS software. This is one of a number of protocols in the pipeline for performing small particle calibration using the fcmpass software package.

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KEYWORDS

fcmpass, flow cytometry, calibration, EVs

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PARENT PROTOCOLS

In steps of

FCMPASS Protocol Collection

MATERIALS TEXT

FCMPASS software can be accessed at https://nanopass.ccr.cancer.gov.

DISCLAIMER:

This protocol summarizes key steps for a specific type of assay, which is one of a collection of assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization. By using the FCMPASS software you agree to the following terms and conditions.

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1 If fluorescence calibration is being performed click the '+' button to add a calibration parameter to the table. If fluorescence calibration is not required, click 'Next'.

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1.1 If you have not yet added the MESF reference bead information that will be used for calibration into the Catalogue', click 'Catalogue' in the top menu bar and complete as per the protocol.

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2 Once a parameter is added double click the ‘Reference Fluorophore’ item and select the bead set used for calibration. The displayed sets are those that have previously been added to the Catalogue.

3 Double click the parameter to select the associated parameter with the correct fluorophore.

4 Double click the relevant cell in the 'New Parameter Name' column to adjust how the calibrated parameter’s name will appear once written to the fcs file.

5 The reference bead values for the selected parameter should appear in the 'Regression Values' table.

6 Click in the 'Acquired Value' box next to each bead reference value and input the acquired statistic

7 Repeat steps 1 to 5 for any further parameters that need to be calibrated. To change the 'Ref Value' table to other fluorophores select them in the reference 'Fluorescence Calibration Parameters' table.

8 Once completed click ‘Next’.

The regression plots for the inputted fluorescence calibration parameters can be checked at any time using the ‘Check Regression(s)’ button. The ‘Advanced Settings’ button can be used to specify an fluorophore:protein ratio or alter the regression method between linear, log, weighted linear, weighted log.

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