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FCMPASS - Cataloguing fluorescence reference materials V.2

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bnimmcc6

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol outlines the steps required to catalogue fluorescence reference materials 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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PROTOCOL CITATION

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fcmpass, flow cytometry, calibration, EVs

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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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Definitions: The term “SOFTWARE” throughout this agreement means the machine readable, binary, object code form, and the related documentation for FCMPASS, a software package that is designed to allow flow cytometer calibration for small particles. The term “RECIPIENT” means the party that downloads the software. The term “PROVIDER” means the National Cancer Institute (NCI), a participating institute of the National Institutes of Health (NIH), and an agency of the United States Government.By downloading or otherwise receiving the SOFTWARE, RECIPIENT may use the SOFTWARE subject to RECIPIENT’s agreement to the following terms:

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Opening the Bead Catalogue

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1 Open FCMPASS

FCMPASS 3

2 Click ‘Catalogue’ in the top menu bar

Bead Catalogue Fluorescence Overview

3 Under the ‘Fluorescence’ tab entry fields exist for each of the pertinent metadata for reporting with fluorescence calibration.

3.1 Enter the name of the fluorophore on the fluorescence reference beads.

3.2 Enter the manufacturer, catalogue number, and lot number fields appropriately.

3.3 In the ‘Bead Ref Values’ field enter each fluorescence beads reference values. This may be in molecules of equivalent soluble fluorophore, equivalent reference fluorophore, or antibody binding capacity.

3.4 After each reference value click the ‘+’ button. To remove a reference value, select the reference value from the listbox below and press the '-' button.

3.5 Once all fields and reference values have been added, press the ‘Create Set’ button. The beads will then appear in the table below as a new row and will be available for selection when performing fluorescence calibration.

4 The 'Delete Set' button is also available to delete any unwanted entries. Simply select the rows that are unwanted and press the 'Delete Set; button.

5 The 'Append Set' button allows for the editing of already existing entries. By selecting the desired row, the information is written into the entry fields above. This information can be modified as needed, and then the 'Append Set' button can be pressed to append the selected table entry.

Note: You can only append a single table entry at a time.

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