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FCMPASS - Cataloguing light scatter reference materials

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1 Works for me dx.doi.org/10.17504/protocols.io.bhvuj66w

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ABSTRACT

This protocol outlines the steps required to catalogue light scatter 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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38548

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.

Terms & Conditions of use for FCMPASS software.

Citation: Joshua A Welsh, Jennifer Jones (08/03/2020). FCMPASS - Cataloguing light scatter reference materials. https://dx.doi.org/10.17504/protocols.io.bhvuj66w

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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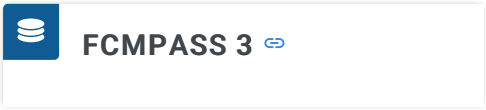
1 Open FCM_{PASS}.



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2 Click ‘Catalogue’ in the top menu bar

3 Under the ‘Light Scatter’ tab entry fields exist for each of the pertinent metadata for reporting with light scatter calibration.

3.1 Diameter CV should be the percent coefficient of variation of the mean diameter provided on the certificate of analysis

3.2 Refractive Index should be the provided refractive index of the bead population on certificate of analysis

> If a refractive index is not available an approximate guide for polystyrene refractive index is 1.59 at 589 nm. Silica tends to vary more in refractive index than polystyrene but tends to be ~1.45 at 589 nm.

3.3 ‘RI Measurement Wavelength’ is the wavelength at which the refractive index was measured and should be provided on the certificate of analysis. This tends to be 589 nm.

3.4 Composition can be selected as polystyrene, silica, or other. If polystyrene or silica are selected, changes in detection wavelength e.g. 488 nm to 405 nm are accounted for using the appropriate Sellmeier equations. If ‘Other’ is selected then the refractive index change is made propositionally to the sheath refractive index.

3.5 Manufacturer, Catalogue Number, and Lot Number should all be completed appropriately.

4 Once the fields have been completed for a bead population click ‘Add Bead’. The population should then appear in the table below.

5 Once the relevant beads have been added ‘Bead Sets’ can be created. A bead set are the bead populations that are used for calibration. Any number of bead sets and combinations can be made.

5.1 In the ‘Selection’ column of the table, check all the bead populations to be included within a bead set.

5.2 Click ‘Create Set’, enter a unique Set name, and click ‘OK’.

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6 Once your bead set has been defined you will be able to perform light scatter calibration.

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