Aug 03, 2020

FCMPASS - Fluorescence calibration

DOI
dx.doi.org/10.17504/protocols.io.bhvyj67w

Joshua A Welsh[1], Jennifer Jones[1]

[1]Translataional Nanobiology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health

Translational Nanobiolo...

Jennifer Jones
NIH

DOI: https://dx.doi.org/10.17504/protocols.io.bhvyj67w

Protocol Citation: Joshua A Welsh, Jennifer Jones 2020. FCMPASS - Fluorescence calibration. **protocols.io https://dx.doi.org/10.17504/protocols.io.bhvyj67w**

Manuscript citation:
Welsh J A, Jones J C,Small Particle Fluorescence and Light Scatter Calibration Using FCMPASSSoftware,Current Protocols in Cytometry, 94, e79. doi: 10.1002/cpcy.79

Protocol status: Working
We use this protocol and it's working

Created: June 24, 2020

Last Modified: June 29, 2023

Protocol Integer ID: 38552

Keywords: fcmpass, flow cytometry, calibration, EVs, fluorescence calibration this protocol, fluorescence calibration parameter, fluorescence calibration, fcmpass software package, fcmpass software, fcmpass, performing small particle calibration, small particle calibration, calibration parameter, calibration this protocol,

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.

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:

1. THE SOFTWARE SHALL NOT BE USED IN THE TREATMENT OR DIAGNOSIS OF HUMAN SUBJECTS. RECIPIENT is responsible for compliance with all laws and regulations applicable to the use of the SOFTWARE.
2. RECIPIENT shall not distribute the SOFTWARE, in whole or in part without express advance written approval of PROVIDER.
3. The SOFTWARE may be used for research, academic, and educational purposes only. The SOFTWARE may not be used for commercial purposes. RECIPIENT will not license or sell or use the SOFTWARE for commercial purposes or applications.
4. The SOFTWARE that is distributed pursuant to this Agreement has been created by United States Government employees. In accordance with Title 17 of the United States Code, section 105, the SOFTWARE isnot subject to copyright protection in the United States. Other than copyright, all rights, title and interest in the SOFTWARE shall remain with the PROVIDER.
5. RECIPIENT shall not modify, extend, decompile, make derivatives of, merge, publish, reverse engineer or distribute the SOFTWAREwithout written permission from PROVIDER.
6. RECIPIENT may publish or otherwise publicly disclose the results of using the SOFTWARE. RECIPIENT agrees to acknowledge PROVIDER’s contribution of the SOFTWARE in all written publications containing any data or information regarding or resulting from use of the SOFTWARE.
7. THE SOFTWARE IS PROVIDED "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND NONINFRINGEMENT ARE DISCLAIMED. IN NO EVENT SHALL THE PROVIDER OR THE INDIVIDUAL DEVELOPERS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS

SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE. PROVIDER makes no representations that the use of SOFTWARE will not infringe any patent or proprietary rights of third parties.

8. RECIPIENT may, on an as-needed basis, send to PROVIDER reports regarding the application of the SOFTWARE and the effectiveness and problems encountered in using the SOFTWARE, without disclosing RECIPIENT's confidential information. Information from general reports may be used by the PROVIDER to enhance the capabilities of the SOFTWARE. Reports can be forwarded to the PROVIDER at one of the following addresses: joshua.welsh@nih.gov or jennifer.jones2@nih.gov
9. No indemnification for any loss, claim, damage, or liability is intended or provided by either Party under this Agreement. Each Party shall be liable for any loss, claim, damage, or liability that said Party incurs as a result of said Party's activities under this Agreement, except that Provider, as an agency of the United States, assumes liability only to the extent as provided under the Federal Tort Claims Act (28 U.S.C. Chapter 171 Sections 2671-2680).
10. RECIPIENT agrees not to claim, infer, or imply endorsement by the United States Government, or any of its organizational units, contractors or employees. RECIPIENT agrees not to use any trademarks, service marks, trade names, logos or product names of NCI or NIH to endorse or promote products derived from the SOFTWARE without specific, prior and written permission.
11. Title in the SOFTWARE shall remain with the PROVIDER. It is understood that nothing herein will be deemed to constitute, by implication or otherwise, the grant to either party by the other of any license or other rights under any patent, patent application or other intellectual property right or interest. PROVIDER reserves the right to distribute the SOFTWARE to others and to use it for PROVIDER's own purposes. The United States Government explicitly retains all rights to use the SOFTWARE for any purpose, to have it used on the Government's behalf or to allow others to use it.

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.

Materials

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

Troubleshooting

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'.

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.

.

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’.

Note

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.