## Application Note No. 994-4006

# Xcyto® 5

## GFP Expression Assay: Fast and robust quantification of GFP expression using the Xcyto® 5 system

**Product description**

The Xcyto® 5 image cytometer and XcytoView™ software integrate image acquisition, image analysis, feature extraction and data presentation in one step for seamless quantitative data of individual cells and cell populations within heterogenous samples.

**Application**

This Application note describes a protocol for easy quantification of Green Fluorescent Protein (GFP) expression and may among others be used for determination of transfection efficiency. Xcyto® 5 is optimized to have a high dynamic range for assays with both dim and bright signals (e.g. using the human CMV promoter) and can be used for a wide range of cell types, such as animal and yeast cells.

**Introduction**

GFP is widely used as a reporter for gene expression. Using an appropriate promoter, GFP can be expressed in the cells by itself or attached to the protein of interest as a fusion protein.

In order to quantify GFP expression in live cells, a suspension of cells transfected/transformed with GFP is stained with a cell membrane-impermeable dye, such as DAPI, to stain non-viable cells, followed by imaging and analysis using the Xcyto® 5 image cytometer and the XcytoView™ software.

---

**Procedure**

The following protocol was developed using mammalian, insect, and fission yeast cells, but can be adapted for most cell types. Growth medium, cell density, cell type and other factors may influence labelling. We recommend testing a concentration range of DAPI to establish optimal conditions for your preferred cell model.

Materials needed

- GFP transfected/transformed cells in suspension [1, 2]
- Complete medium for the cell type used [1]
- DAPI (500 µg/ml, Solution 12, Cat. No. 910-3012)
- OPTIONAL: phosphate buffered saline (PBS) [1]
- Xcyto® 2-Chamber Slide Type 100-A (Cat.no. 942-0010)

[1] Provided by the user

[2] An untreated control sample should be included. Preferable, use logarithmically proliferating cells as control

Protocol

1. For each sample, harvest cells, determine cell concentration, and aliquot a representative sample from the cell suspension to a tube (e.g. Eppendorf)
2. NOTE: If comparing two or more samples, it is important that they are at the same concentration ($2{\times}10^6$ cells/ml recommended)
3. Add DAPI to a final concentration of 1 µg/ml to stain non-viable cells
4. Mix thoroughly by pipetting or gently vortexing
5. Analyze immediately or within 15 minutes for best results
   NOTE: Cells can be imaged directly in medium containing stain, no wash steps are required
6. Load the Xcyto® 2-Chamber Slide Type 100-A with 50 µl of cell suspension
7. Engage Xcyto® 5 image cytometer by opening XcytoView™ Software
8. Eject sample tray and place slide on tray
9. Select the *GFP Expression* protocol in the Protocol Browser or drop-down menu; both are available from the Main Window
10. In the data acquisition control section in the main window, select your sample media, enter sample ID, and press the *Run* icon
    OPTIONAL: Create user-defined stop criteria in the acquisition limits section, available in the main window, by the number of either counts or views. Criteria can be edited when limits are disabled
    a. To define a gate to use for the counts limit, wait for the file to be displayed in the plot manager, create a gate or use a template-defined gate, select the name in the drop-down menu and enable the counts limit
11. Cells are imaged, segmented and analyzed automatically. Numerical cell data is presented in the plot manager
    NOTE: Inspection of the gates, “Cells” and Q1, is crucial and may require adjustment for proper analysis

During image acquisition and analysis, results will be displayed in the Plot Manager when the analysis of the first view is complete. For every analyzed view, result data are added incrementally to the results displayed.

Cells are identified based on transmitted light microscopy. Percentages of GFP negative, GFP positive and dead cells can be read from the density plot. A quadrant gate marks the cell population of each sample (see examples below).

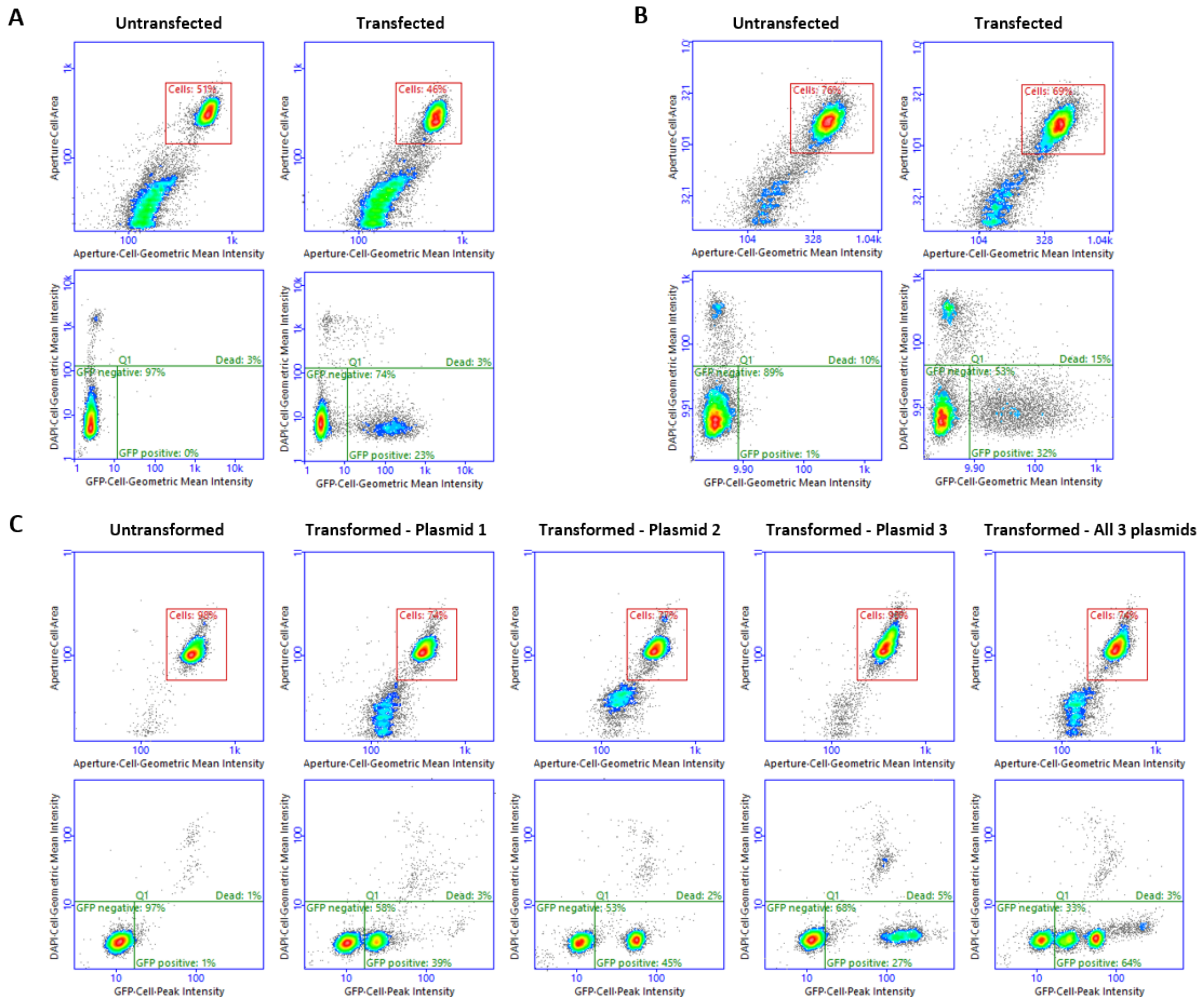


***Figure 1.*** *24 hours after transfection/transformation with a GFP harboring plasmid, cells were harvested, stained with DAPI according to the protocol, imaged and analyzed with the Xcyto® 5 image cytometer and XcytoView™ software using the* GFP Expression *protocol. Untransfected/untransformed cells were included as control. Aperture masking plots display identified cells based on transmitted light microscopy and the gate "Cells". The density plots show the GFP intensity versus the intensity of DAPI. The "Cells" population were gated on in the density plots. Quadrants, in the density plots, were used to demarcate the various cell populations. Inspection of the gates, "Cells" and "Q1", is crucial and may require adjustment for proper analysis. Note: Only single cells are included in the analysis.* ***A)*** *U2OS cells.* ***B)*** *S2 insect cells.* ***C)*** *Fission yeast cells transformed with dim, intermediate and bright GFP expressing plasmids.*

**Notes**

This assay has been optimized to relatively high expression levels of GFP, such as the level reached when using human CMV promotor. We have noticed that cells which express very high levels of GFP may cause neighboring cells to falsely appear GFP positive, resulting in an overestimation of the transfection/transformation efficiency. This problem occurs mainly when having a combination of strongly GFP-expressing cells AND high cell concentrations. Thus, if the GFP signal is extremely bright make sure that the cell concentration is $1 \times 10^6$ cells/ml or below.

For samples containing small cell types or particles, such as PBMCs, we recommend a settling time of 2-5 minutes after loading the Xcyto® slides to avoid movement of sample components during data acquisition. Movement during data acquisition can cause errors in image overlay and thereby quantification of cell data.

Analysis time is highly correlated with cell density of the sample. Thus, lower cell densities increase acquisition and analysis times.

**Handling and storage**

For handling and storage of ChemoMetec® instruments, reagents and Xcyto® slides refer to the corresponding product documentation. For other reagents, refer to the material data sheet from the manufacturer of the reagents and chemicals.

**Warnings and precautions**

For safe handling and disposal of the ChemoMetec® reagents and Xcyto® slides refer to the corresponding product documentation and the Xcyto® Software User Guide. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

**Limitations**

The Xcyto® 5 system, consisting of the Xcyto® 5 image cytometer and XcytoView™ software, is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the Xcyto® 5 system depend on correct use of the reagents, Xcyto® slides and the Xcyto® 5 instrument and might depend on the type of cells being analyzed. Refer to the Xcyto® 5 Software User Guide for instructions and limitations.

**Liability disclaimer**

This application note is for RESEARCH PURPOSES ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

**Product disclaimer**

ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

**Copyright**

Copyright © ChemoMetec A/S 2020. All rights reserved. No part of this publication and referred documents may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written consent of ChemoMetec A/S, Gydevang 43, DK-3450 Allerod, Denmark.

ChemoMetec and Xcyto are registered trademarks owned by ChemoMetec A/S. XcytoView is a trademark of ChemoMetec A/S. All other trademarks are the property of their respective owners.