APPLICATION NOTE

Measuring marker expression with imaging cytometry on a plate reader

Introduction

The presence or absence of cellular proteins can indicate a reaction to stimulation, a state of differentiation, unique characteristics of a specific cell type, or success of a gene transfection. For example, the surface expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) is usually absent from normal vascular endothelium but can be stimulated *in vitro* by the cytokine TNF-α to elicit an inflammatory response. Such protein expressions in a cell can be readily visualized and easily quantified using imaging cytometry.

Quantitative measurement of marker expression in cells

Human umbilical vein endothelial cells (HUVEC) were cultured in 96-well plates for 48 hours and then treated for 2 hours with anti-inflammatory compounds, SB202 and SB203, at varying doses. Following the treatment, VCAM-1 expression was stimulated with TNF-α for 24 hours. Cells were then fixed using 4% paraformaldehyde and stained with anti-VCAM fluorescein-conjugated antibodies. Images were acquired using SpectraMax® MiniMax™ Imaging Cytometer, a field upgrade option for the SpectraMax® i3 Multi-Mode Detection Platform. Image analysis was performed on-the-fly using the Marker Expression Protocol of SoftMax® Pro Software. Compound efficacy was evaluated with dose-response curve in SoftMax Pro Software, showing whether the anti-inflammatory compounds were successful in protecting the cells from cytokine stimulation, thus inhibiting subsequent VCAM-1 expression.

The level of VCAM-1 expression was quantitated by setting an intensity threshold to filter out low background fluorescence and a size threshold to filter out artifacts tiny artifacts such as scratches, dust, or bubbles. Figure 1 shows the visually guided setup of the Marker Expression Protocol in SoftMax Pro Software.

Fixed cells stained with fluorescein-conjugated anti-VCAM are identified with the Marker Expression Protocol. Since cells are nearly confluent, the protocol is set up to measure overall marker expression in the field of view instead of segmenting individual cells.

Benefits

- **Expanded plate reader applications with imaging cytometer functionality**
- **Easily measure marker expression levels per cell or per well**
- **Follow a familiar microplate reader workflow**
- **Confidence in the data output with cell visualization**

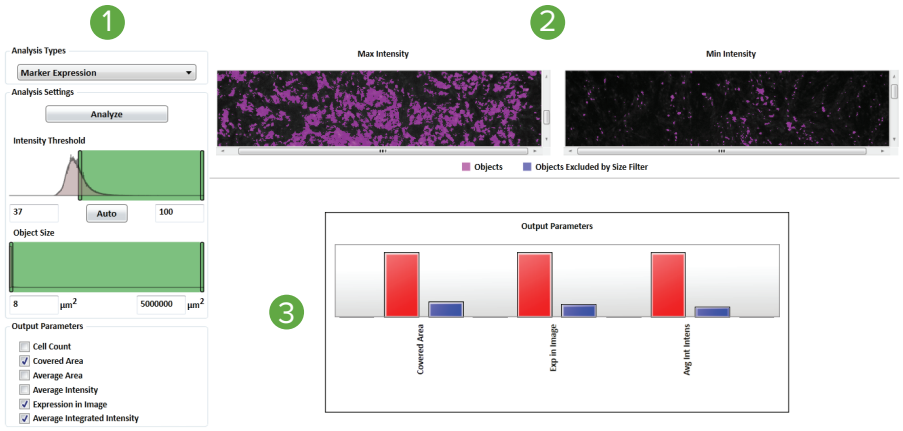

Figure 1. Test analysis parameters on positive and negative samples before plate read. The SoftMax Pro Software interface has several visual components to help set up optimal parameters for image segmentation: **1:** Adjust background intensity and object size thresholds to identify fluorescent cells. **2:** Visualize resulting segmentation masks of positive and negative wells to see if further adjustment is needed. **3:** Choose output parameters that yield large differences between positive and negative wells as seen in bar graphs. The Marker Expression Protocol is used to identify cell areas labeled with the fluorescein-conjugated anti-VCAM antibody. The presence of VCAM-1 indicates a positive inflammatory response.

More information from your marker expression assays

The SpectraMax MiniMax Imaging Cytometer provides visualization and information beyond simple fluorescence intensity in the well. Measured areas of fluorescence are localized to the cells expressing the markers-of-interest and the large imaged field-of-view allows a significant number of cells to be interrogated at once. Intuitive analysis using SoftMax Pro Software allows plotting of dose response curves and calculation of IC_{50} values. Taken together, the MiniMax Imaging Cytometer simplifies cellular imaging and provides results that complement standard plate reader assays for measuring marker expression in cells.

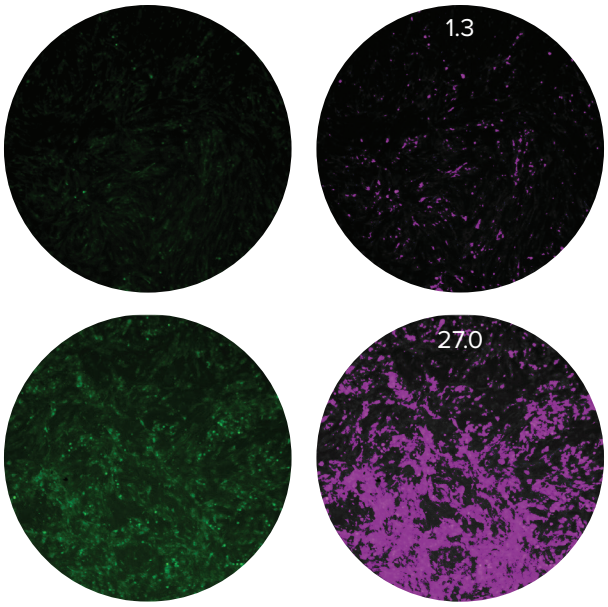

Figure 2. Cellular response to treatment with anti-inflammatory compounds. Images on the left show cells with fluorescent VCAM-1 expression, plus purple masks on the right show segmentation of cells exhibiting the inflammation marker. Cells treated with a high dose of the anti-inflammatory compound SB202 **(top row)** show little VCAM-1 expression while cells treated with a very low dose of SB202 **(bottom row)** were stimulated by the cytokines and expressed a large quantity of VCAM-1. Values are given in normalized total signal intensity.

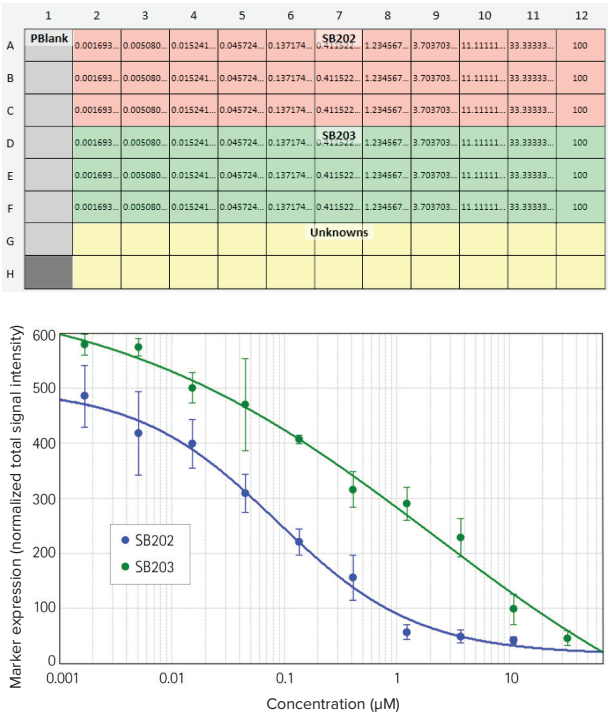

Figure 3. Analysis of marker expression results. Top: Annotated wells in the plate template. **Bottom:** A plot of the mean marker expression vs. compound concentration. As concentration of the anti-inflammatory compounds increases, amount of cytokine stimulated VCAM-1 expression decreases. The normalized total signal intensities can be plotted with one of many curve-fit options and IC_{50} concentrations can be determined. SB202 IC_{50}= 0.1 µM, SB203 IC_{50}=1.8 µM.

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com
Check our website for a current listing of worldwide distributors.

4/16 0120-1822B
Printed in USA

MOLECULAR DEVICES