PRODUCT DATA SHEET

IncuCyte® FabFluor-488 Antibody Labeling Reagents for Live-Cell Immunocytochemistry

Catalog numbers 4743, 4744, and 4745

Introduction

The IncuCyte® FabFluor-488 Antibody Labeling Reagents for cell surface marker analysis are supplied as lyophilized solids in sufficient quantity to label 50 µg of test antibody, when used at the suggested molar ratio (1:3 of test antibody to labeling Fab). The lyophilized solid should be stored at 2-8°C (stable for at least 1 year). Once re-hydrated, it is recommended the solution is used as soon as possible or aliquoted and stored at -80°C; avoid freezing and thawing (stable for at least 1 year post re-hydration).

IncuCyte® Opti-Green background suppressor is supplied as 200 µL of 100 mM stock solution in water. The stock liquid should be stored at 2-8°C (stable for at least 1 year). Bring to room temperature and mix well (vortex) before use. Dilute on day of use in assay media.

Background and intended use

IncuCyte FabFluor-488 Antibody Labeling Reagents are designed for quick, easy labeling of Fc containing test antibodies with a green fluorophore. Once labeled the IncuCyte FabFluor-488-antibody complex, in combination with IncuCyte Opti-Green background suppressor can be used for identification of surface expressed antigens in live-cells. In the absence of expressed specific antigen, little or no signal is seen on the cells. In combination with IncuCyte Opti-Green and the IncuCyte® integrated analysis software, background fluorescence is minimized. This reagent has been validated for use with a number of different antibodies in a range of cell types. The IncuCyte® live cell analysis platform enables real-time, kinetic evaluation of live-cell immunocytochemistry.

Recommended use

We recommend that IncuCyte FabFluor-488 Antibody Labeling Reagents are prepared at stock concentrations of 0.5 mg/mL by the addition of 100 µL of sterile water and triturate (not supplied, centrifuge if solution not clear). This will re-hydrate the powder to result in a buffer of 0.01 M sodium phosphate, 0.25 M NaCl at pH 7.6 with 15 mg per mL BSA (IgG and protease free). The reagent may then be diluted directly into the labeling mixture with test antibody. Do not sonicate the solution.

We recommend that IncuCyte Opti-Green background suppressor is mixed well before use. Stock solution should be diluted in complete growth media to produce a final assay concentration of 0.5 mM or a 1 in 200 dilution of stock. This has been shown to be suitable across a range of cell types.

Please see the relevant protocol published on our website:

Essenbioscience.com/Immunocytochemistry

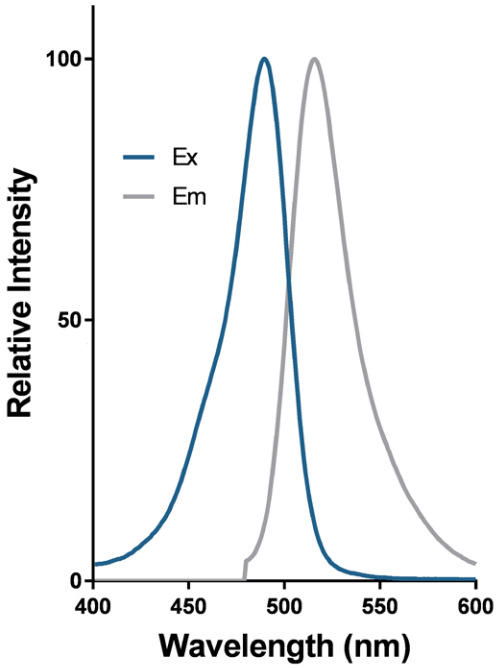


Figure 1. Excitation and emission spectra for IncuCyte FabFluor-488, determined in PBS (pH 7.4).

IncuCyte® A SARTORIUS BRAND

Additional information

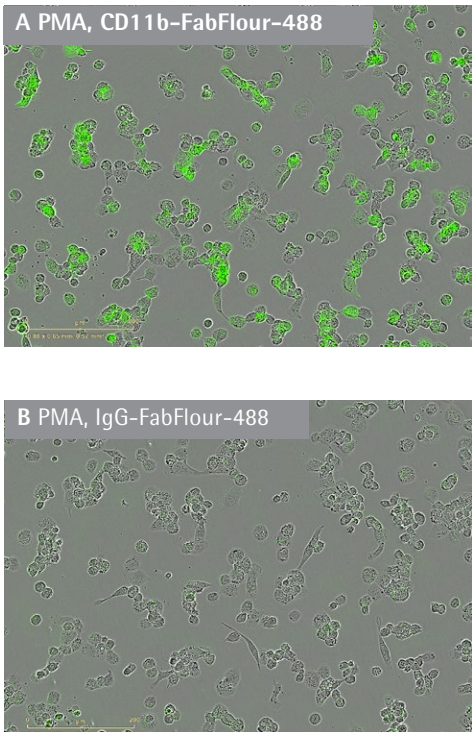
The antibody was purified from antisera by a combination of papain digestion and immunoaffinity chromatography using antigens coupled to agarose beads. Fc fragments and whole IgG molecules have been removed.

Based on antigen-binding assay and/or ELISA the antibody reacts with the Fc portion of mouse IgG1, 2a or 2b but not the Fab portion of mouse immunoglobulins. No antibody was detected against mouse IgM or against non-immunoglobulin serum proteins. The antibody may cross-react with other mouse IgG subclasses or with immunoglobulins from other species.

The SDS can be found on our website:

Essenbioscience.com/immunocytochemistry

Product	Cat No.	Amount	Labeling Suitability	Use
IncuCyte® Mouse IgG2a FabFluor-488 Antibody Labeling Reagent	4743	50 µg	Mouse IgG2a Fc containing Antibody	For use with antibodies containing mouse IgG2a Fc region
IncuCyte® Mouse IgG2b FabFluor-488 Antibody Labeling Reagent	4744	50 µg	Mouse IgG2b Fc containing Antibody	For use with antibodies containing mouse IgG2b Fc region
IncuCyte® Mouse IgG1 FabFluor-488 Antibody Labeling Reagent	4745	50 µg	Mouse IgG1 Fc containing Antibody	For use with antibodies containing mouse IgG1 Fc region



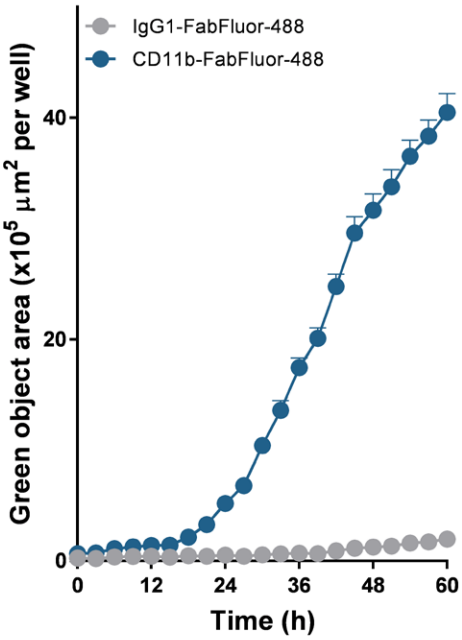


Figure 2. Use of Live-cell immunocytochemistry to quantify real-time increase in CD11b surface expression induced by differentiation of THP-1 cells with PMA (100 nM). α-CD11b and IgG isotype control were labeled with IncuCyte FabFluor-488 using the IncuCyte® Live-cell Immunocytochemistry protocol. THP-1 cells were incubated with 100 nM PMA, to induce differentiation to a macrophage morphology in combination with IncuCyte Opti-Green (0.5 mM) and IncuCyte FabFluor-488-α-CD11b or IncuCyte FabFluor-488-IgG (1 µg/mL). HD phase and green fluorescence images were captured on IncuCyte® S3, every 3 hours over 60 hours using a 20x magnification. Images of cells treated with PMA show green fluorescence in the presence of labeled CD11b (images shown at 48 h) (A). Cells treated with labeled isotype control display no cellular fluorescence (B). The graph shows the quantification of green fluorescence area over time, indicating an increase in CD11b expression in response to differentiation of THP-1 cells with PMA.