SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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PRODUCT DATA SHEET

IncuCyte® FabFluor-pH Red Antibody Labeling Reagents for Antibody Internalization **Catalog numbers 4722, 4723, 4737, 4750, and 4751**

Introduction

The IncuCyte® FabFluor-pH Red Antibody Labeling Reagents for Antibody internalization are supplied as lyophilized solids in sufficient quantity to label 100 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).

Background and intended use

IncuCyte® FabFluor Antibody Labeling Reagents are designed for quick, easy labeling of Fc containing test antibodies with a pH-sensitive fluorophore. The pH-sensitive dye based system exploits the acidic environment of the lysosomes to quantify internalization of the labeled antibody. As FabFluor labeled antibodies reside in the neutral extracellular solution (pH 7.4) they interact with cell surface specific antigens and are internalized. Once in the lysosomes, they enter an acidic environment (pH 4.5 – 5.5) and a substantial increase in fluorescence is observed. In the absence of expression of the specific antigen, no internalization occurs and the fluorescence intensity of the labeled antibodies remains low. With 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 System enables real-time, kinetic evaluation of antibody internalization.

Recommended use

We recommend that IncuCyte® FabFluor-pH Red Antibody Labeling Reagents are prepared at a stock concentration of 0.5 mg per 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 pH7.6 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.

Please see the relevant protocol published on our website:
Essenbioscience.com/Antibody

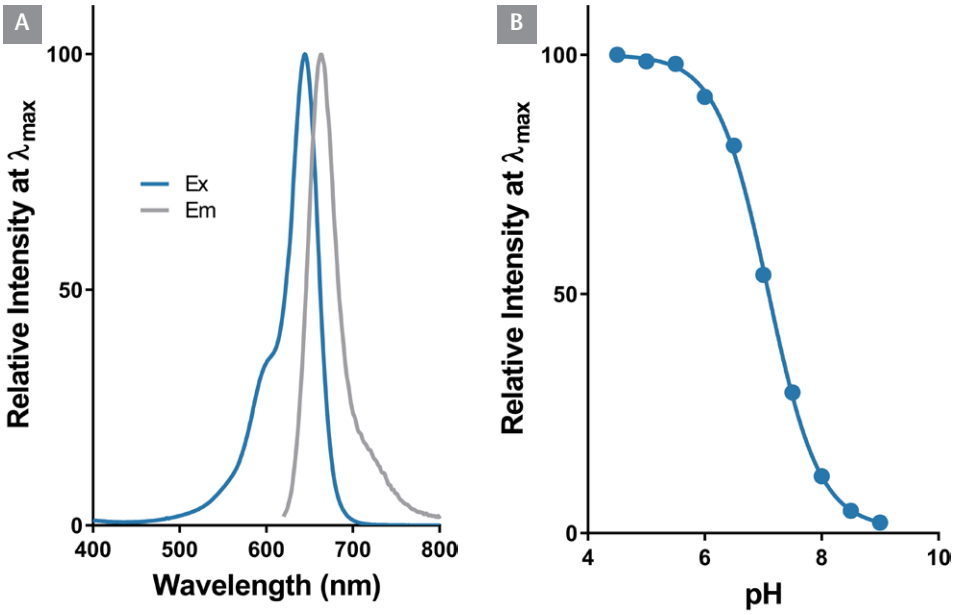


Figure 1. pH sensitivity of FabFluor.
(A) Excitation and emission spectra for the FabFluor-pH sensitive dye determined in pH 4.5 buffer.
(B) Curve to demonstrate pH sensitivity of probe.

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.

Human (Cat #4722) - Based on immunoelectrophoresis and/or ELISA the antibody reacts with the Fc portion of human IgG heavy chain but not the Fab portion of human IgG. No antibody was detected against human IgM, IgA or against non-immunoglobulin serum proteins. The antibody may cross-react with other immunoglobulins from other species.

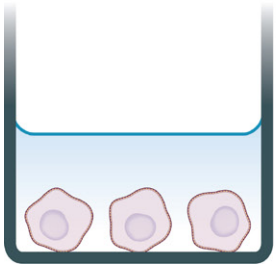
Mouse IgG1 (Cat #4723), IgG2a (Cat #4750) or IgG2b (Cat #4751) - Based on antigen-binding assay and/or ELISA the antibody reacts with the Fc portion of mouse IgG, IgG2a or IgG2b, respectively, but not the Fab portion of mouse immunoglobulins. No antibody was detected against mouse IgM or against non-immunoglobulin serum proteins. The antibody may cross-react with other mouse IgG subclasses or with immunoglobulins from other species.

Rat (Cat #4737) - Based on immunoelectrophoresis and/or ELISA the antibody reacts with the Fc portion of rat IgG heavy chain but not the Fab portion of rat IgG. No antibody was detected against rat IgM, IgA or against non-immunoglobulin serum proteins. The antibody may cross-react with other immunoglobulins from other species.

Safety Data Sheets can be found on our website: **Essenbioscience.com/Antibody**

Quick Guide

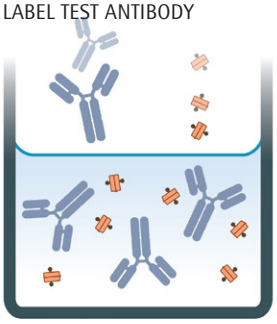
1 SEED CELLS



Cell seeding

Seed cells (50 µL/well, 5,000-30,000 cells/well), into 96-well plate and leave to adhere (2-24 h, depending on cell type).

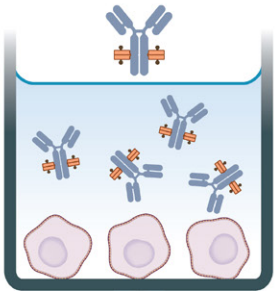
2 LABEL TEST ANTIBODY



Labeling of test antibody with IncuCyte® FabFluor Reagent

Mix antibody and FabFluor Reagent at a molar ratio of 1:3 in media, 2x final assay concentration. Incubate for 15 minutes to allow conjugation.

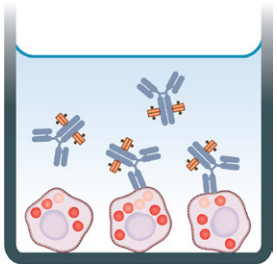
3 ADD TO CELLS



IncuCyte® FabFluor-labeled antibody addition

Add antibody-FabFluor mix (50 µL/well) to cell plate.

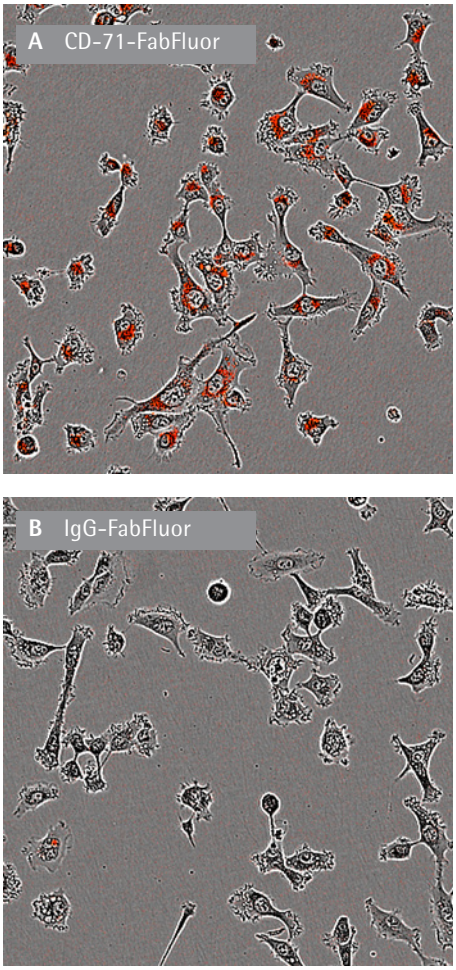
4 LIVE-CELL FLUORESCENT IMAGING



Automated imaging and quantitative analysis

Capture images every 15-30 minutes (10x or 20x) in IncuCyte® for 24-48 hours. Analyze using integrated software.

Example data



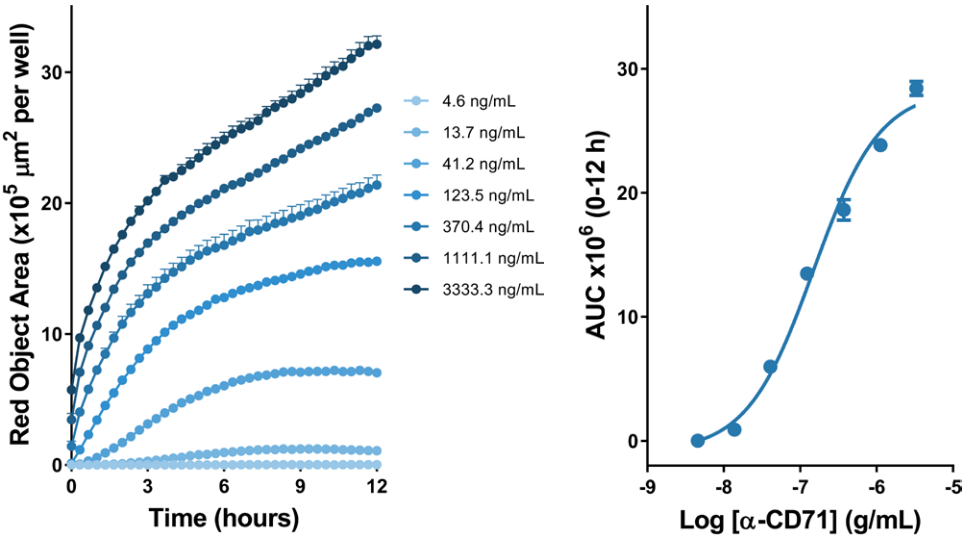


Figure 2. Concentration-dependent increase in antibody internalization of IncuCyte® FabFluor labeled-α-CD71 in HT1080 cells.
α-CD71 and IgG1 isotype control were labeled with IncuCyte® FabFluor using the above protocol. HT1080 cells were treated with either FabFluor-α-CD71 or FabFluor-IgG1 (4 µg/mL), HD phase and red fluorescence images were captured every 30 minutes over 12 hours using a 10x magnification. Images of cells treated with FabFluor-α-CD71 display red fluorescence in the cytoplasm (images shown at 6 h) **(A)**. Cells treated with labeled isotype control display no cellular fluorescence **(B)**. **(C)** Time-course of FabFluor-α-CD71 internalization with increasing concentrations of FAbFluor-α-CD71 (progressively darker symbols). Internalization has been quantified as the red object area for each time-point. **(D)** Concentration response curve to FabFluor-α-CD71. Area under the curve (AUC) values have been determined from the time-course shown in panel A (0-12 hours) and are presented as the mean ± SEM, n=3 wells.

Product	Cat No.	Amount	Use
IncuCyte® Human FabFluor-pH Red Antibody Labeling Reagent	4722	50 µg	For use with antibodies containing human Fc region
IncuCyte® Mouse IgG1 FabFluor-pH Red Antibody Labeling Reagent	4723	50 µg	For use with antibodies containing mouse IgG1 Fc region
IncuCyte® Rat FabFluor-pH Red Antibody Labeling Reagent	4737	50 µg	For use with antibodies containing rat Fc region
IncuCyte® Mouse IgG2a FabFluor-pH Red Antibody Labeling Reagent	4750	50 µg	For labeling antibodies containing mouse IgG2a Fc region
IncuCyte® Mouse IgG2b FabFluor-pH Red Antibody Labeling Reagent	4751	50 µg	For labeling antibodies containing mouse IgG2b Fc region

Product label license

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