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Antibody labeling reagents to rapidly screen for binding, internalization, and degradation of biologics and antibody drug conjugates

Ryan Holly, Yi-Zhen Hu, Aimei Chen, & Chris Langsdorf. Thermo Fisher Scientific. 29851 Willow Creek Rd. Eugene, OR 97402

Introduction

There is a growing need in the immunotherapy field for tools to rapidly screen for novel targeted antibodies, whether looking for cells expressing IgG's, screening for target binding, or monitoring trafficking, internalization, and degradation. Thermo Fisher Scientific has developed next generation reagents to enable high-throughput antibody screening, working across a variety of sample types including primary B cells and hybridoma's, while being compatible with a wide range of platforms including flow cytometry, high content imaging, and Incucyte.

Figure 1: Antibody labeling methods

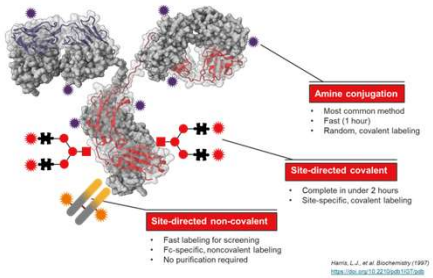
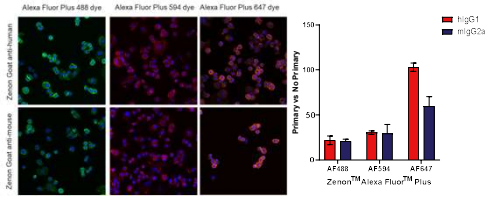
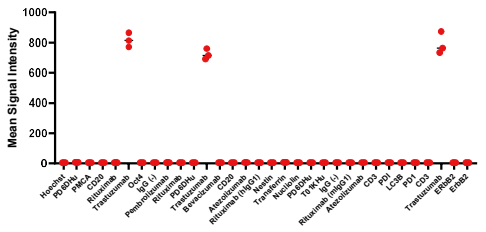


Figure 2: Invitrogen™ Zenon™ Alexa Fluor™ Plus IgG Labeling Reagents allow for rapid screening of therapeutic antibodies using high content screening platforms such as the Thermo Scientific™ CellInsight™ CX7 LZR Pro HCS Platform



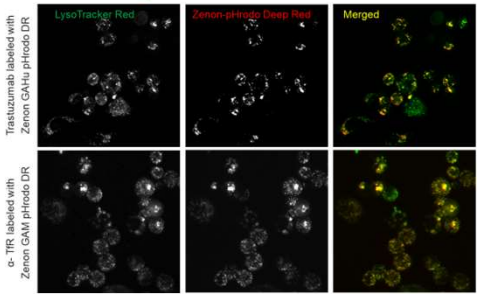
Her-2 positive SKBR3 cells were treated with trastuzumab labeled with Zenon Alexa Fluor Plus Labeling Reagents for 30 minutes at 37°C and 5% CO2. Cells were washed 3x with 1x PBS and imaged with the CellInsight CX7 LZR Pro high content imaging platform. Background was determined by incubating cells with Zenon reagents without trastuzumab.

Figure 3: Screen of primary antibodies for cell surface binding to SKBR3 cells using Zenon Alexa Fluor Plus IgG Labeling Reagents



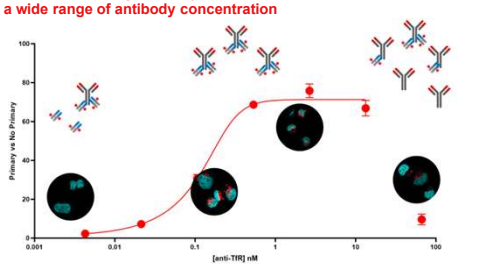
Various mouse and human IgG's, were treated with Zenon Goat anti-Human Alexa Fluor Plus Labeling Reagent. Following a 5-minute incubation, SKBR3 cells were treated with antibodies for 60 minutes and then screened on the CellInsight CX7 LED Pro HCS platform for positive binding. Trastuzumab was the only antibody in this screen known to bind to the cell surface of SKBR3 cells.

Figure 4: Invitrogen™ Zenon™ pHrodo™ Deep Red IgG Labeling Reagents for screening antibody internalization, fluoresce only upon late endosomal and lysosomal localization



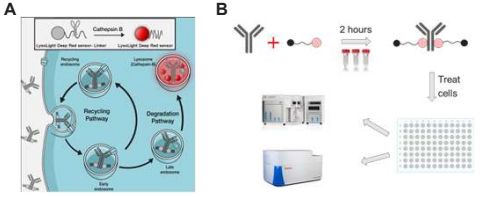
SKBR3 cells were first treated with either Zenon Goat anti-Human pHrodo Deep Red labeled trastuzumab or Zenon Goat anti-Mouse pHrodo Deep Red labeled anti-transferrin receptor for 16 hours to allow for complete internalization. Prior to imaging, cells were treated with 50 nM Invitrogen™ LysoTracker™ Red for 1 hour. Cells were imaged on the Invitrogen™ EVOS™ M7000 imaging system using RFP and Cy5 filter cubes.

Figure 5: Zenon pHrodo Deep Red IgG Labeling Reagents are sensitive, allowing antibody screens for positive internalization over a wide range of antibody concentration



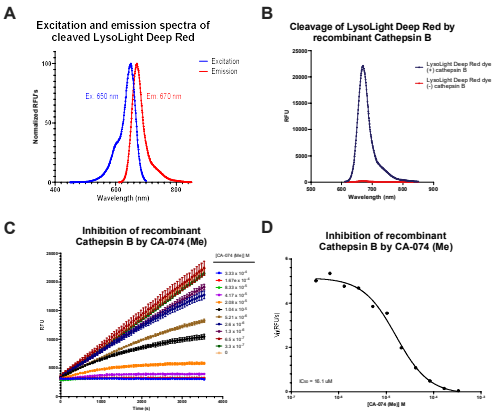
Five-fold serial dilutions of α-transferrin receptor were prepared from 10 ng/mL to 0.64 pg/mL while keeping Zenon Goat anti-Mouse pHrodo Deep Red labeling reagent constant at a final assay concentration of 200 nM. After 5-minute labeling, SKBR3 cells were treated with labeled antibodies. Following 16 - hour internalization at 37°C and 5%CO_2 cells were imaged and quantified on the CellInsights CX7 LZR Pro HCS imaging system.

Figure 6: Invitrogen™ LysoLight™ Deep Red sensor: A first in class sensor to directly monitor lysosomal degradation of target proteins



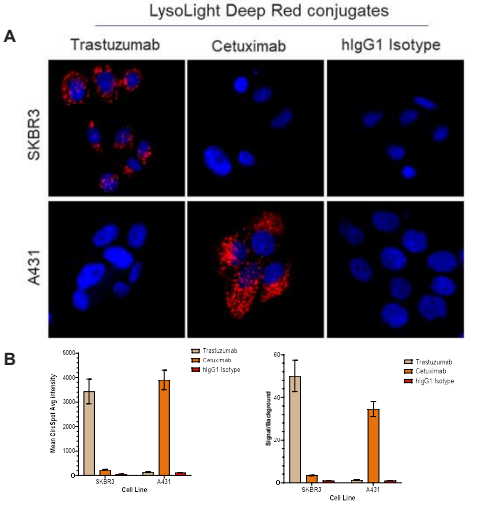
(A) LysoLight Deep Red is a sensor that directly monitors cathepsin B activity in cells. In its un-cleaved form, LysoLight Deep Red has no background fluorescence, and only upon cleavage by the lysosomally localized protease, cathepsin B, is the dye cleaved leading to bright fluorescence in the Cy5 channel. (B) The sensor contains an SDP ester allowing it to be conjugated to lysine residues on antibodies or other proteins of interest in order to monitor the degradation of that protein.

Figure 7: LysoLight Deep Red sensor is sensitive and specific for recombinant cathepsin B



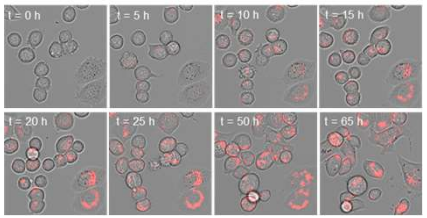
(A) Excitation and emission spectra of LysoLight Deep Red. (B) LysoLight Deep Red dye treated with or without 5 nM recombinant cathepsin B for 2 hours and fluorescence emission scan was measured in a multimode fluorescence plate reader. (C) 5 nM cathepsin B was titrated with the cathepsin B inhibitor CA-074 (Me) over a range of concentrations for 1 hour followed by incubation with LysoLight Deep Red dye to monitor cathepsin B activity. (D) Initial velocities were plotted against inhibitor concentration to determine the IC_{50} value of 16.1 µM.

Figure 8: LysoLight Deep Red labeled antibodies are antigen specific



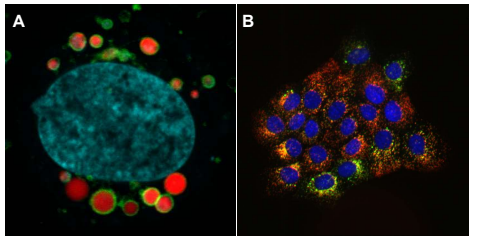
LysoLight Deep Red was conjugated to trastuzumab (Her-2+/EGFR-), cetuximab (Her-2-/EGFR+), or human IgG 1 Isotype control (Her-2-/EGFR-). SKBR3 (+Her-2) and A431 (+EGFR) were treated with conjugates for 16 hours under normal cell culture conditions. Following treatment, cells were labeled with Hoechst dye (blue), imaged for LysoLight Deep Red (red) on the EVOS M7000 (A) and quantified using the CellInsight CX7 LZR Pro (B).

Figure 9: LysoLight Deep Red signal is stable and non-toxic to cells



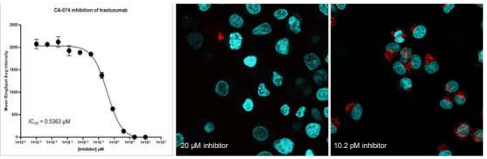
SKBR3 cells were treated with 1 µg/mL trastuzumab-LysoLight Deep Red conjugate for 65 hours. Images were taken every 15 minute on the EVOS M7000 equipped with OSI2 onstage incubator.

Figure 10: LysoLight Deep Red is lysosomal specific



(A) SKBR3 cells were treated with CellLights Lysosome GFP (green) for 24 hours followed by treatment with trastuzumab-LysoLight Deep Red (red) conjugate for 16 hours. Before imaging on Zeiss LSM 980, cells were treated with Hoechst (cyan) (B) A431 cells were treated with cetuximab-LysoLight Deep Red conjugate for 16 hours (red), then stained with LysoTracker Red (green) and Hoechst (blue) for 30 minutes and imaged on the EVOS M7000.

Figure 11: LysoLight Deep Red sensors are specific for lysosomal activity



SKBR3 cells were treated with the cathepsin B inhibitor CA-074 (Me) for 2 hours followed by trastuzumab-LysoLight Deep Red conjugate for 16 hours. Signal intensity was quantified on the CellInsights CX7 LZR Pro and images taken on Zeiss LSM 980 confocal.

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