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Antibody labeling reagents to rapidly screen for the binding and internalization of therapeutic antibodies

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Abstract

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 and internalization. Thermo Fisher Scientific has developed the next generation of Zenon 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.

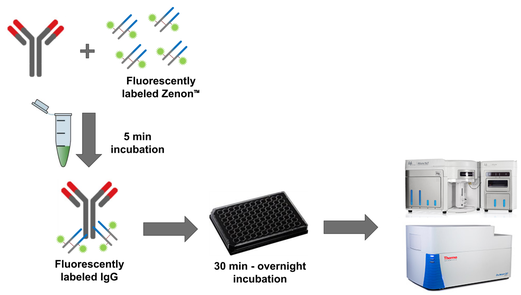
Introduction

The Invitrogen™ Zenon™ Alexa Fluor™ Plus IgG Labeling Reagents to screen for antibody binding provide a fast, versatile, and reliable method to evaluate antibody binding to cell surface antigens. Zenon screening reagents are labeled with Invitrogen™ Alexa Fluor™ Plus 488, Alexa Fluor™ Plus 594, or Alexa Fluor™ Plus 647 dyes (i.e., labeling reagent). The labeling reagent is directed against the Fc portion of an intact mouse or human IgG primary antibody. The formation of the Zenon–antibody complex is rapid and requires no purification steps (Figure 1).

The Invitrogen™ Zenon™ pHrodo™ Deep Red Labeling Reagents for screening antibody internalization provides a highly sensitive method for monitoring antibody internalization. Zenon pHrodo Deep Red utilizes the same screening reagent as Zenon Alexa Fluor Plus reagents, replacing Alexa Fluor dyes with the pH-sensitive dye, pHrodo™ Deep Red dye. The pHrodo Deep Red sensors have minimal fluorescence at neutral pH and only upon entry into the late endosome and lysosome do they brightly fluoresce. Zenon pHrodo Deep Red Labeling Reagents are perfectly suited for screening the endocytosis and degradation of targeted antibodies including antibody-drug conjugates.

Materials and Method

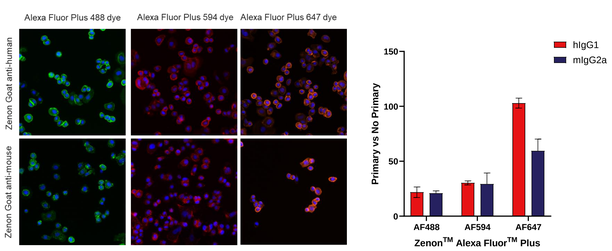
Figure 1: Zenon IgG labeling reagents allow for simple, fast, and purification free labeling of all subclasses of mouse or human antibodies



Mouse or human IgG's are labeled with Zenon labeling reagents for 5 minutes at room temperature. Cells are treated with labeled IgG's for as little as 30 minutes to detect surface antigens and up to 24 hours to look at antibody internalization. Imaging and quantification can then be detected and analyzed by flow cytometry, high content imaging, or live cell incubators.

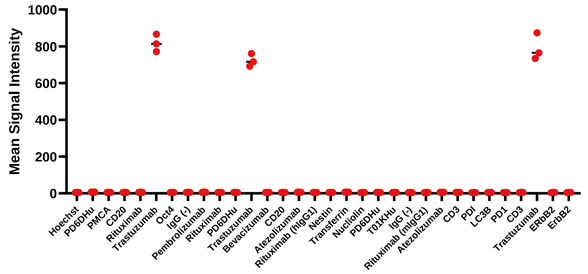
Results

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



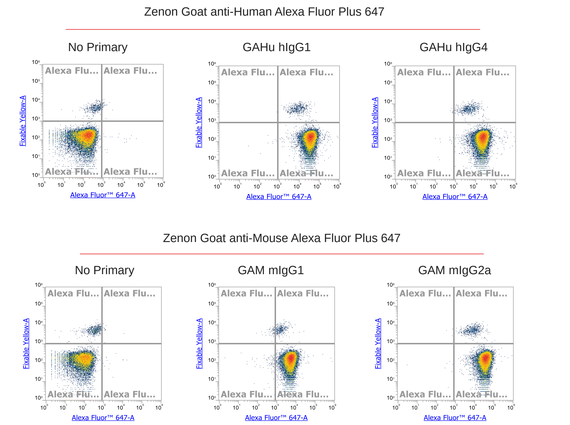
Her-2 positive SKBR3 cells were treated with Trastuzumab labeled Zenon Alexa Fluor Plus Labeling Reagents for 30 minutes at 37ºC and 5% CO2. Cells were washed 3x with 1x PBS and imaged with the CellInsight CX7 LZR Pro high content imaging platform. Background was determined by incubating cells with Zenon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

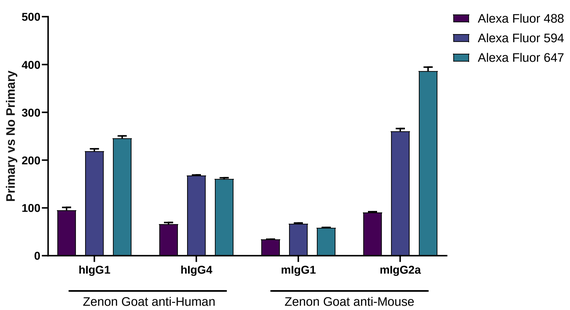
Figure 3: Screen of primary antibodies for cell surface binding to SKBR3 cells using Zenon Alexa Fluor Plus IgG Labeling Reagents



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 CellInsights CX7 LED Pro for positive binding. Trastuzumab was the only antibody in this screen known to bind to the cell surface of SKBR3 cells.

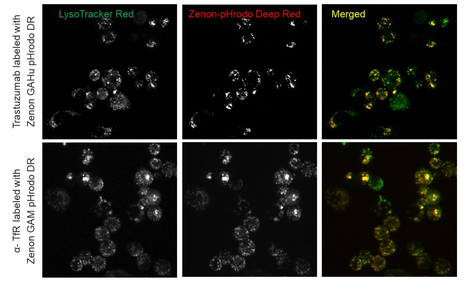
Figure 4: Zenon Alexa Fluor Plus IgG Labeling Reagents efficiently detect human or mouse antibody subclasses by flow cytometry.





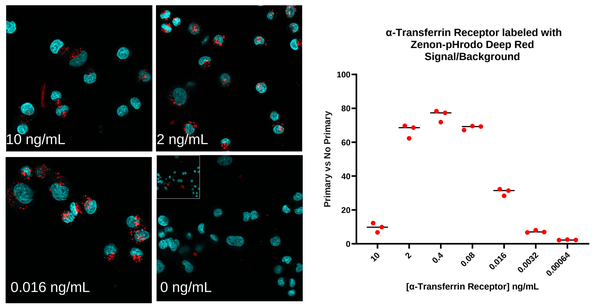
Various human or mouse anti-CD20 subclasses were labeled with Zenon Goat anti-Human or Goat anti-Mouse Labeling Reagents respectively. Ramos cells were treated with labeled antibodies for 60 minutes, washed with PBS, and stained with live/dead before running on the Invitrogen™ Attune™ NxT flow cytometer. Background was determined by cells treated with Zenon reagent without primary antibody.

Figure 5: Invitrogen Zenon pHrodo Deep Red IgG Labeling Reagents for screening antibody internalization, fluoresce only upon late endosomal and lysosomal localization



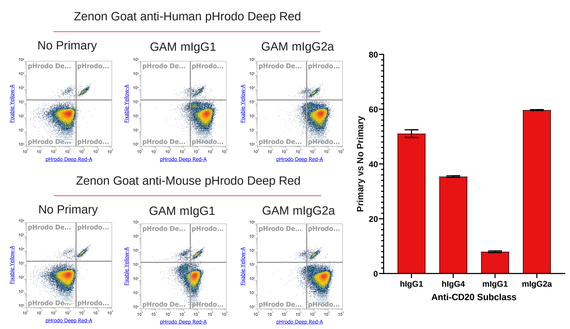
SKBR3 cells were first treated with either Zenon Goat anti-Human pHrodo Deep Red labeled Trastuzumab or Zenon Goat anti-Mouse pHrodo Deep Red labeled anti-transferrin receptor for 16 hours to allow for complete internalization. Prior to imaging, cells were treated with 50 nM Invitrogen™ LysoTracker™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

Figure 6: Invitrogen Zenon pHrodo Deep Red IgG Labeling Reagents are sensitive allowing antibody screens for positive internalization over a wide range of antibody concentration



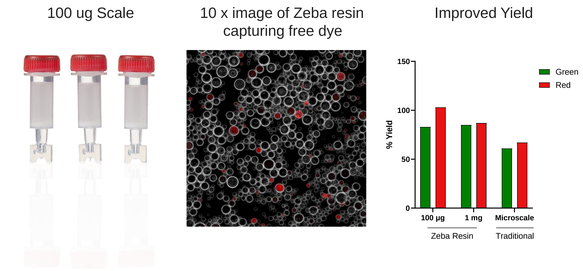
Five-fold serial dilutions of α-Transferrin receptor were prepared from 10 ng/mL to 0.64 pg/mL while keeping Zenon Goat anti-Mouse pHrodo Deep Red Labeling Reagent constant at a final assay concentration of 200 nM. After 5-minute labeling, SKBR3 cells were treated with labeled antibodies. Following 16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

Figure 7: Invitrogen™ Zenon™ pHrodo™ Deep Red IgG Labeling Reagents are well suited for flow cytometry applications.



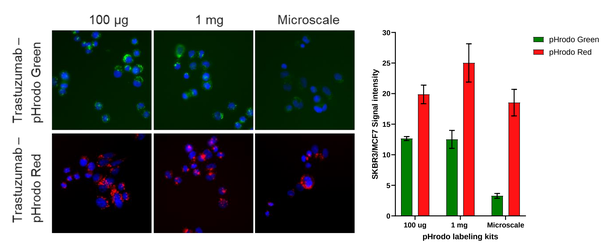
Various human or mouse IgG subclasses were labeled with Zenon Goat anti-Human or Zenon Goat anti-Mouse pHrodo Deep Red Labeling Reagents respectively. Following 16-hour internalization with Ramos cells, cells were treated with DAPI and screened for cells positive for pHrodo Deep Red on the Attune NxT flow cytometer. Background was determined by cells treated with Zenon reagent without primary antibody.

Figure 8: New Invitrogen™ pHrodo™ Red and Green Antibody Labeling Kits using Invitrogen™ Zeba™ Dye and Biotin Removal columns for rapid and efficient purification of labeled antibodies



pHrodo Red and Green Antibody Labeling Kits come in two sizes utilizing Zeba Dye and Biotin Removal columns allowing for purification in under 5 minutes. The resin utilizes a combination of a 7k molecular weight cutoff as well as ion exchange properties allowing for near complete removal of small molecular weight dyes from antibody conjugation reactions.

Figure 9: Improved signal to background in cells treated with Trastuzumab – pHrodo Green or Trastuzumab – pHrodo Red



Trastuzumab was reacted with pHrodo Red iFL or Green iFL – STP ester for 2 hours followed by purification with Zeba Biotin and Dye Removal columns or purification from the legacy microscale kits. SKBR3 (positive for Her-2) and MCF7 (negative for Her-2) cells were treated with antibody conjugates for 16 hours at 37ºC, 5% CO_2. Cells were imaged and quantified on CellInsights CX7 LED

Conclusions

Thermo Fisher Scientific has developed sensitive and rapid Zenon reagents to allow for high throughput antibody screening. These tools not only greatly improve the workflow from our existing Zenon reagents, but allow for increased sensitivity allowing the field to make decisive decisions when selecting pre-clinical candidate immunotherapies.

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