Creative Biolabs®
Antibody-drug Conjugate

ADC Case Study

Antibody Internalization Assay

Introduction

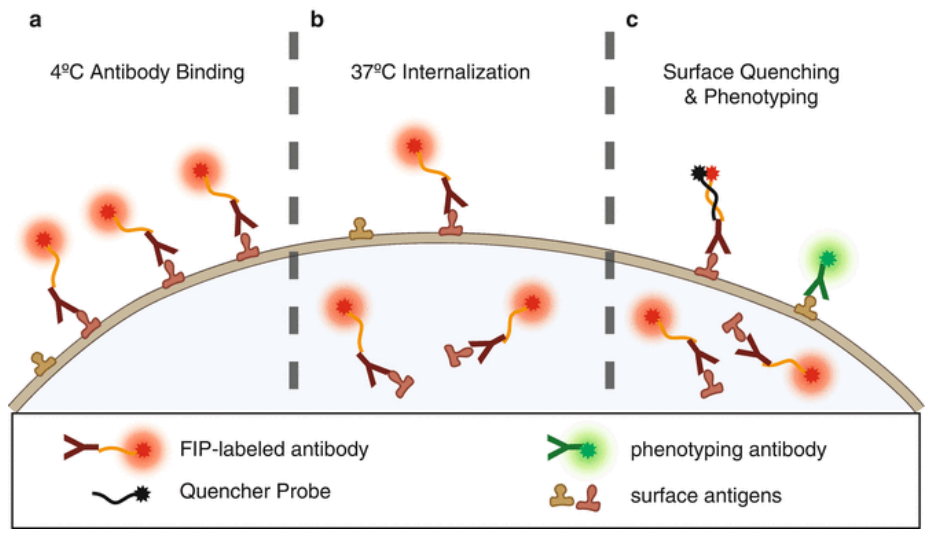
Antibodies against cell surface antigens can be internalized through their specific interactions with these proteins. The anti-cancer efficacy of antibody associated therapeutics like antibody-drug conjugates is thought to rely on their uptake by cancer cells expressing the surface antigen. Therefore, the measurement of internalization efficacy of an antibody is critical for its curative effect. Based on years of experiences in antibody-drug-conjugate (ADC) developing and characterization, Creative Biolabs provides internalization analysis service to help clients verify the internalization of their antibody and ADC samples, and this service can be also very useful in helping clients select appropriate internalizing antibody for immunotherapy development.

Presented here is a case study for the antibody internalization detection via the confocal microscopy and flow cytometry strategies.

Internalization: An important process during antibody-based therapeutics

The efficacy of certain antibody-based therapeutics depends on internalization process. Once internalization, such as the ADC molecules are transported to subsequent intracellular compartments where the toxic payload drug is released to accomplish the cytotoxicity of the ADC. Thus, the rate and extent of antibody internalization is of crucial importance to its effectiveness and need to be characterized precisely.

CBL has adopted and perfected several techniques to study antibody/ADC internalization. Confocal microscopy and flow cytometry are suitable methods to detect the internalization efficiency.



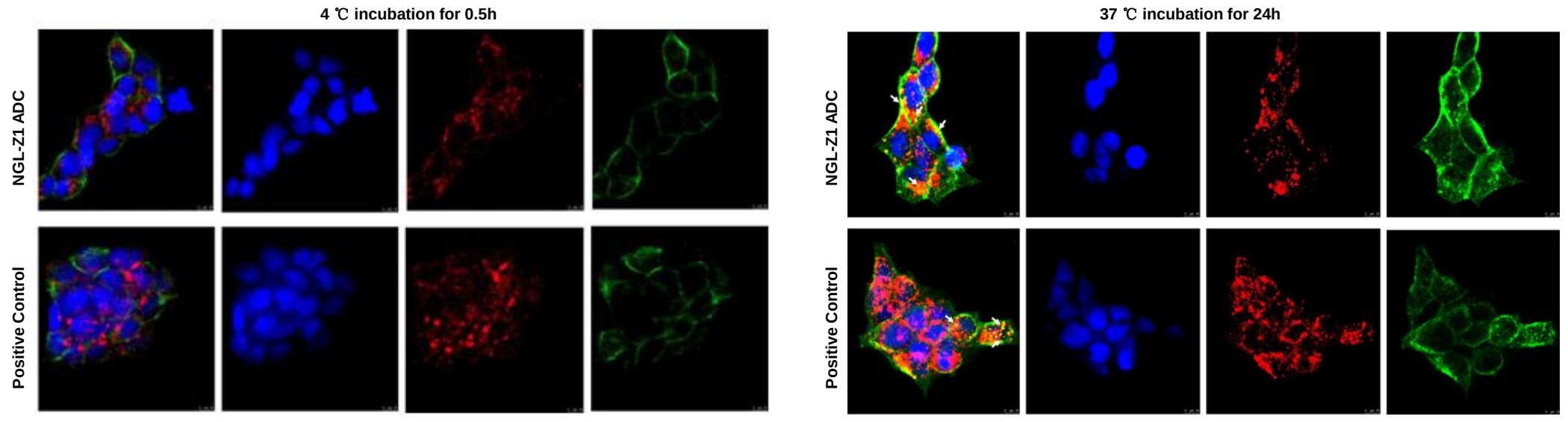
Case 1: Internalization and intracellular trafficking of anti-HER2 mAb-vc-Toxin (NGL-Z1 ADC)

Measurement method:

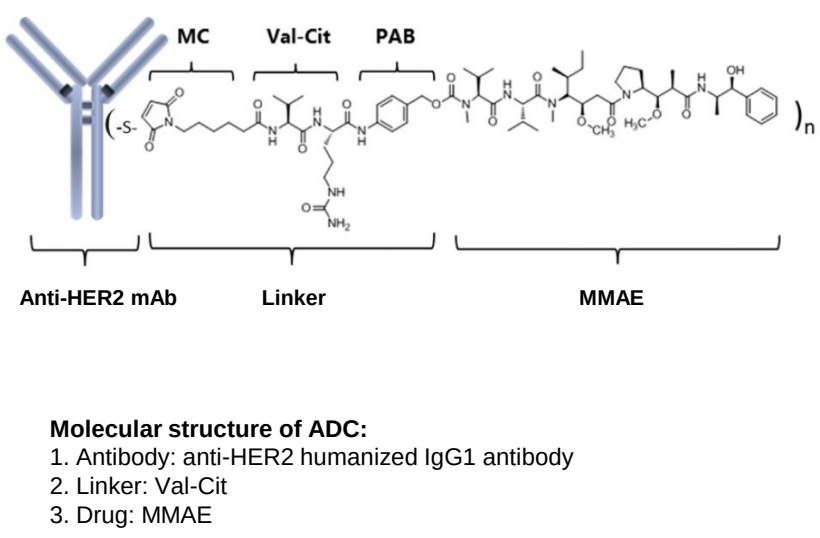
1. Cell were seeded per plate and incubated with NGL-Z1 ADC and positive control antibody 30 min at 4 ℃ and incubated 24h at 37 ℃, respectively.
2. For internalized NGL-Z1 ADC, the cells were incubated with Alexa Fluor 488-labeled goat anti-human IgG, then incubated with Alexa Fluor 647-Labeled anti-human CD107b antibody, respectively.
3. The nuclear compartment was stained with DAPI. Fluorescence images were taken using confocal microscope.

Results:

NGL-Z1 ADCs or positive control antibody was shown as green, and lysosome markers CD107b was shown as red, and DAPI-stained nuclei was shown in blue. Compared to incubation at 4℃ for 0.5 h, the significant yellow signal appeared at both ADC and positive control antibody groups after 37 ℃ incubation for 24h，and the yellow signal refers to the co-localization of signals for ADC or positive antibody (green)with lysosome markers (red), which indicating that ADC and positive control antibody were internalized and transported to the lysosomes successfully.



Case 2: Cellular internalized and lysosome trafficking of anti-HER2 mAb and anti-HER2 mAb-vc-MMAE



Molecular structure of ADC:
1. Antibody: anti-HER2 humanized IgG1 antibody
2. Linker: Val-Cit
3. Drug: MMAE

Measurement method:

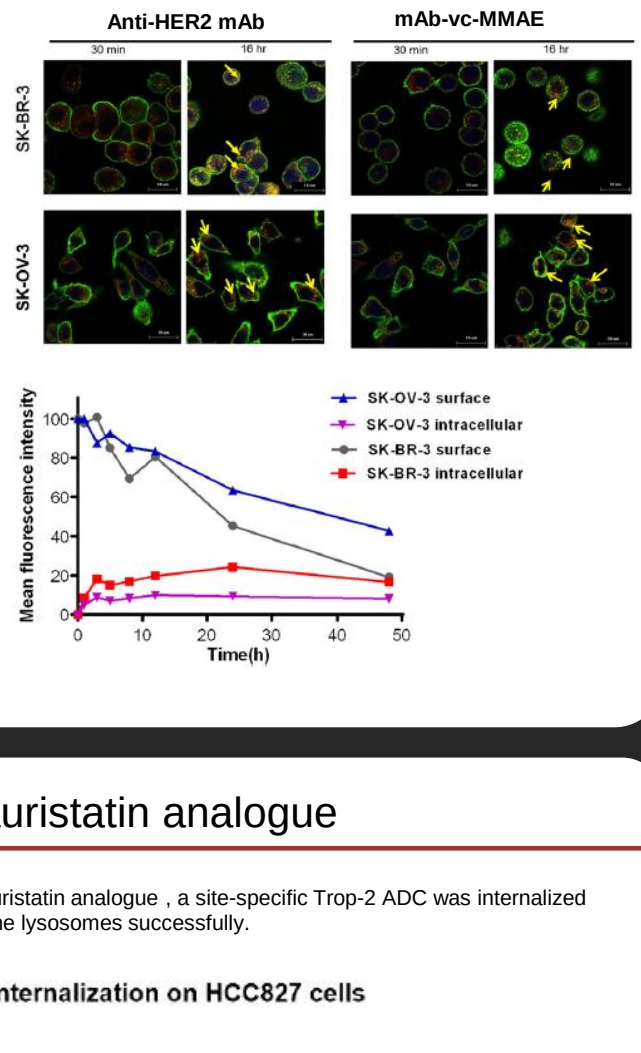
1. Cell were seeded per plate and incubated with mAb-vc-MMAE 30 min on ice and incubated at 37 ℃.
2. For internalized mAb-vc-MMAE, the cells were incubated with mouse anti-human lamp-1, lamp-2, and CD63, then incubated with Alexa Fluor 488-labeled goat anti-human IgG and Alexa Fluor 568-Labeled goat anti-mouse IgG, respectively.
3. The nuclear compartment was stained with DAPI. Fluorescence images were taken using a TCS confocal microscope.
4. The cells were collected and analyzed by flow cytometry using FACS Calibur:

(a) % of MFI *tx*=MFI of sample incubated at 37 ℃/MFI of control sample incubated at 4 ℃ × 100.

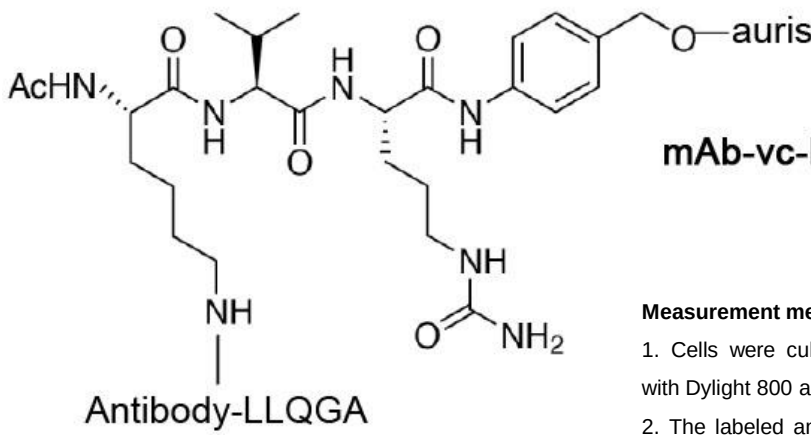
(b) Internalization percentage (% *tx*) of cell surface-bound antibodies = 100 - % of MFI *tx*.

Results:

Anti-HER2 mAb and mAb-vc-MMAE were internalized and transported to the lysosomes successfully.



Case 3: Internalization kinetics and intracellular trafficking of anti-Trop mAb-vc-auristatin analogue



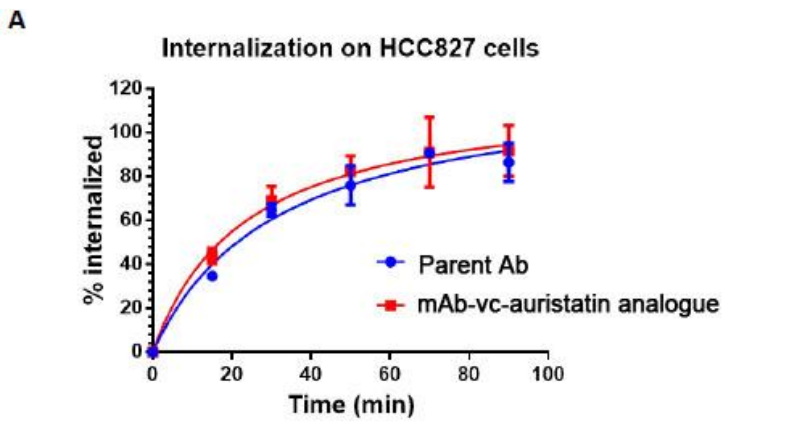
Molecular structure of ADC:
1. Antibody: anti-Trop-2 hIgG1 antibody
2. Linker: Val-Cit
3. Drug: auristatin analogue

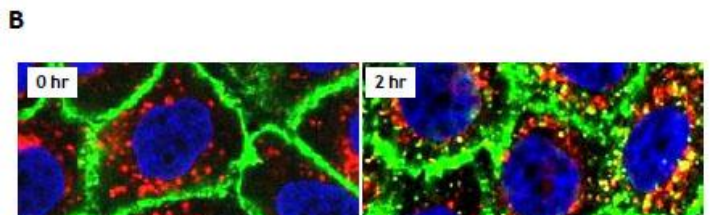
Measurement method:

1. Cells were cultured, harvested, primary antibody was labeled with Dylight 800 antibody labeling kit.
2. The labeled antibody was added to the cells and incubated at 4℃.
3. To initiate internalization, cells were pre-warmed binding buffer supplemented with unlabeled parental Ab and incubated in a 37℃.
4. Internalization was stopped by the quench buffer. Cells were treated with trypsin-EDTA supplemented with papain and incubated in a 37℃ to remove un-internalized antibodies on the surface.
5. Samples were collected to measure the maximal amount of surface-bound fluorescence signals.
6. Co-localization of mAb-vc-auristatin analogue with lysosomal marker LAMP-2 was detected with confocal microscopy.

Results:

Anti-Trop mAb-vc-auristatin analogue , a site-specific Trop-2 ADC was internalized and transported to the lysosomes successfully.





Conclusions

1. Using the "Antibody Internalization Assay" custom analysis service, Creative Biolabs successfully detect the antibody or ADC internalization process.
2. "Antibody Internalization Assay" custom service is well-suited for all kinds of antibody-associated therapeutics with only a few steps.
3. The internalization assay is intuitive and quick method to achieve the optimal detection results.

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