Epitope Tag Conjugation System Provides Versatility for Multiplex Assay Design

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Abstract

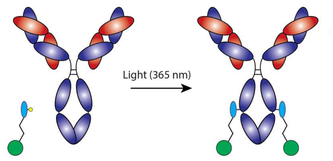
With the ever-growing use of multiplex assays in research, therapeutics, and diagnostics, the need to conjugate antibodies to dyes, enzymes, and other materials is increasing. While there are many challenges to overcome regarding conjugation, the largest hurdle is maintaining antibody activity after conjugation. To combat these conjugation challenges, AlphaThera developed a conjugation system that utilizes a small adapter protein to label antibodies quickly and site-specifically, resulting in antibodies that maintain their activity post conjugation. The adapter protein contains a customizable C-terminal region that can be modified to contain diverse labels including fluorescent dyes, click-chemistry, drugs, and epitope tags. Incorporating the epitope tag conjugation system with Fortis Life Sciences anti-epitope tag detection antibodies, we developed an easy and adaptable system for generating conjugated antibodies to use in multiplex assays.

We used this system to detect staining of multiple markers including CD3, Granzyme B, PCNA, and actin via flow cytometry, IHC, ICC, and western blot. One of the tested antibodies retained normal activity post-conjugation even though prior attempts with metal tagging via sulfhydryl groups resulted in lost activity. The vast array of conjugated anti-epitope tag antibodies allows for the selection of specific dyes/materials that are optimized to your desired assays. Additionally, since the secondary antibody is detecting the epitope tag, multiplexing is simplified, and antibody host is rendered irrelevant. This epitope tag system provides a simple, site-specific conjugation method that has incredible adaptability for any multiplex assay.

Methodology

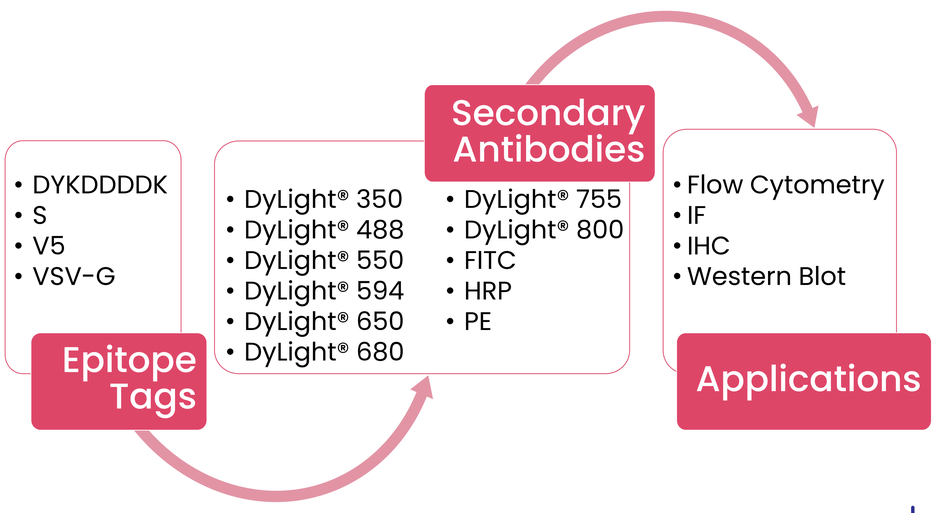
oYo-Link® reagents consist of low molecular weight (~8 kDa), high-affinity antibody-binding domains that possess a photo-crosslinker within their Fc-binding site. Upon illumination with non-damaging Black-light, oYo-Link® forms a covalent bond with the antibody. Any label that is attached to oYo-Link® will be covalently attached to the desired antibody.

Example linker with epitope tags.



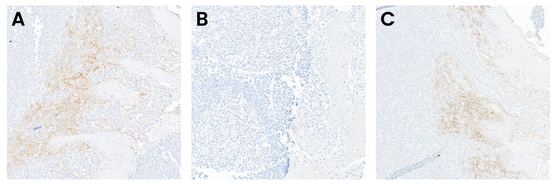
Site specificity of oYo-Link® covalent bonding.

The variety of epitope tags and detection antibodies available enables the use of combinations that are optimized for specific assays.



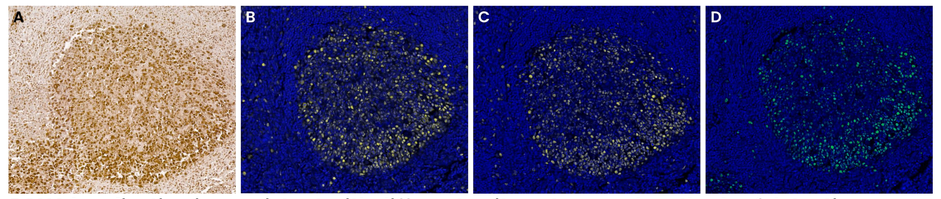
Antibodies retain activity and specificity after epitope conjugation.

Epitope Tagged Antibodies Retain Activity



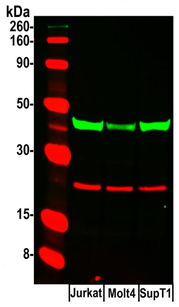
Anti-beta Catenin antibody retains activity after AlphaThera oYo-Link® conjugation.
Tonsil tissue stained with 2.5ug/mL rabbit anti-beta catenin recombinant monoclonal (A700-086) unconjugated (A), conjugated to neodymium (145Nd) via chemical conjugation using sulfhydryls (B), or conjugated to DYKDDDDK via AlphaThera oYo-Link® (C). Secondary: goat anti-rabbit IgG heavy and light chain, highly cross adsorbed antibody HRP conjugated (A120-501P). Detection: DAB.

Concordant staining observed with different epitope tags.

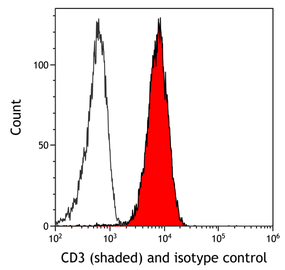


PCNA localization is consistent with different epitope tags and methods of detection.
FFPE tonsil tissue stained with rabbit anti-PCNA recombinant monoclonal (A700-075) conjugated to V5 tag (A,D), VSV-G tag (B), or S tag (C). Secondary: rabbit anti-V5 tag antibody HRP conjugated (A), rabbit anti-VSV-G tag antibody DyLight® 550 conjugated (B), rabbit anti-S tag antibody DyLight® 550 conjugated (C), rabbit anti-V5 tag recombinant monoclonal antibody DyLight® 488 conjugated (D).

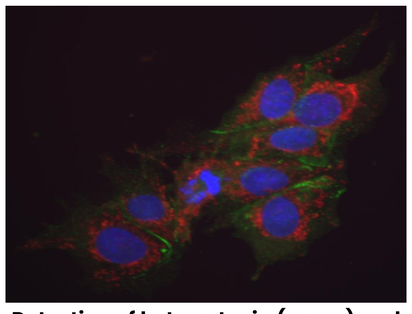
Epitope Tag Conjugation System performs well in multiple assays.



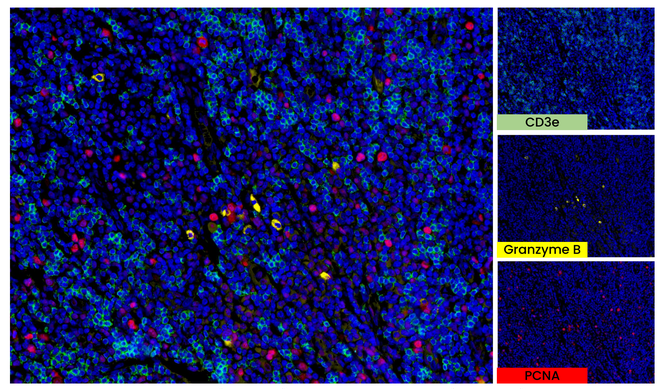
Detection of CD3 (red) and actin (green) by multiplex fluorescent western blot.
Whole cell lysate from Jurkat, Molt4, and SupT1 cells were stained with rabbit anti-CD3e recombinant monoclonal antibody (A700-016) conjugated to DYKDDDDK and rabbit anti-cytoskeletal actin recombinant monoclonal antibody (A700-057) conjugated to VSV-G.



Detection of CD3 by flow cytometry.
PFA fixed and methanol permeabilized Jurkat cells were stained with rabbit anti-CD3e recombinant monoclonal antibody (A700-016) conjugated to DYKDDDDK.



Detection of beta catenin (green) and ATP5A1 (red) by IF.
HepG2 cells grown in chamber slides were fixed. Permeabilized, and then stained with rabbit anti-beta catenin recombinant monoclonal antibody (A700-086) conjugated to DYKDDDDK and rabbit anti-ATP5A1 polyclonal antibody (A304-939A) conjugated to S-tag.



Detection of CD3e (green), Granzyme B (yellow), and PCNA (red) by IHC-IF.
FFPE tonsil tissue was stained with rabbit anti-CD3e recombinant monoclonal (A700-016) conjugated to DYKDDDDK, rabbit anti-Granzyme B recombinant monoclonal (A700-022) conjugated to S-tag, and rabbit anti-PCNA recombinant monoclonal (A700-075) conjugated to VSV-G.

Secondary antibodies used: *rabbit anti-ECS (DYKDDDDK) tag recombinant monoclonal antibody DyLight® 650 conjugated, rabbit anti-S tag antibody DyLight® 550 conjugated, rabbit anti-VSV-G tag antibody DyLight® 550 conjugated., and rabbit anti-VSV-G tag antibody DyLight® 594 conjugated*

Conclusion

Multiplex assays are a common technique used in research, therapeutics, and diagnostics because they can provide a wealth of information from a single sample. Often the success of these assays is dependent on the quality of conjugated antibodies used, which can be costly to acquire and periodically lose antibody activity. To combat these challenges, we have collaborated with AlphaThera to pair their epitope tag conjugation with our anti-epitope tag detection antibodies to create an easy-to-use system that is adaptable to many different multiplex assay formats. Using this system, we were able to show consistent antibody activity after conjugation to multiple epitope tags and across multiple assays. This flexibility of this system allows for the selection of materials optimized for your specific assays.

We would like to acknowledge AlphaThera for providing the oYo-Link® epitope tag conjugation system.