Expanding Multiplexing Capabilities with TrailBlazer™ StarBright™ Dye Antibody Conjugation Kits

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Imaging, western blotting, and flow cytometry multiplexing experiments are often constrained by the availability of antibodies with suitable fluorophores, hindering panel size and optimal experimental design. TrailBlazer Tag and StarBright Dye Label Kits (Figure 1 and Table 1) address this challenge by enabling flexible, high-performance antibody labeling to one of 32 premium StarBright Dyes.

Here we demonstrate the versatility of TrailBlazer Kit–labeled antibodies across multiple applications, including western blotting (Figure 2), confocal microscopy (Figure 3), and flow cytometry (Figures 4 and 5). We show high-quality multiplexing data can be generated, allowing optimal panel design without the use of secondary antibodies.

TrailBlazer Kit–labeled antibodies are stable for up to one year, owing to the novel SpyTag-SpyCatcher technology used. This long-term stability enables reproducible performance and eliminates the need for repeated labeling reactions, making these conjugates particularly well suited to longitudinal studies (Figure 6).

TrailBlazer Tag and StarBright Dye Label Kit Overview

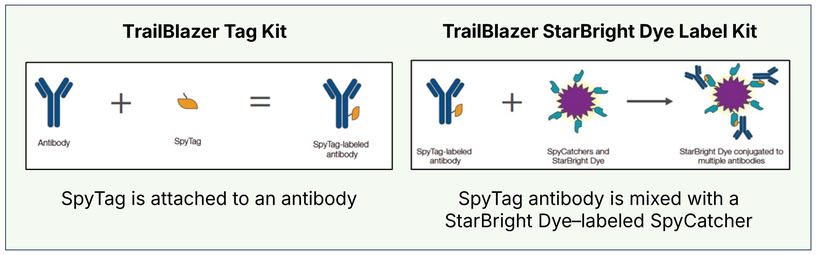

Fig. 1. TrailBlazer Tag and StarBright Dye Label Kit protocol overview.

Range

Table 1. TrailBlazer StarBright Dye Label Kit range.

355 nm	405 nm	488 nm	561 nm	640 nm
SBUV400	SBV440	SBB580	SBY575	SBR670
SBUV445	SBV475	SBB615	SBY605	SBR715
SBUV510	SBV515	SBB675	SBY665	SBR775
SBUV575	SBV570	SBB700	SBY720	SBR815
SBUV605	SBV610	SBB765	SBY800	
SBUV665	SBV670	SBB810		
SBUV740	SBV710			
SBUV795	SBV760			
	SBV790			

Materials and Methods

Western blotting. Cell lysates and protein ladder (#1610373) were separated in reducing conditions using a Stain-Free Criterion Gel (#5678125), transferred onto a LF PVDF membrane (#1704274) and blocked with EveryBlot Blotting Buffer (#12010020). Membranes were probed with a primary antibody for 1 hr at room temperature (RT), washed, and, if required, incubated with a secondary antibody for 1 hr at RT and washed. Images were acquired using a ChemiDoc™ MP Imaging System.

Microscopy. MCF7 (#HTB-22, ATCC) breast cancer cells were cultured in a 24-well plate for 24 hr prior to standard immunostaining. Cells were fixed using 4% paraformaldehyde (PFA) and permeabilized using a 0.1% Triton solution. 10% normal goat serum was used for blocking. 0.5 µg of each TrailBlazer StarBright Dye–conjugated antibody/traditional directly conjugated antibody was used for direct staining. Imaged using a BioTek Cytation C10 confocal imaging reader (Agilent) at 20x magnification. SBY605 and PE were detected with TRITC confocal imaging cube (Ex. 556/20; Em. 600/37) and SBR670 and APC with Cy5 confocal imaging cube (Ex. 635/18; Em. 685/40).

Flow cytometry. Human red cell lysed peripheral blood or peripheral blood mononuclear cells (PBMCs) were blocked with 10% human serum. Cells were incubated with a single antibody or panel (Table 2) for 1 hr at RT. Cells were washed 3x in PBS + 1% BSA. Cells were acquired before or after fixation with different fixatives on a 5-laser ZE5 Cell Analyzer (Bio-Rad Laboratories). Analysis was performed using FCS Express 7 Software (De Novo Software by Dotmatics).

TrailBlazer Kit–Labeled Antibodies in Western Blotting

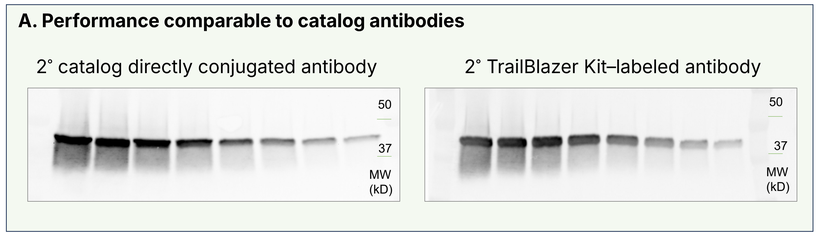

Fig. 2. TrailBlazer Kit–labeled primary and secondary antibodies show high performance in western blotting. HeLa lysate spiked with decreasing amount of maltose binding protein (MBP). **A.** Probed with Mouse Anti-Human MBP detected with Goat Anti-Mouse labeled to StarBright Blue 700 using TrailBlazer Tag Kit (#12020038) and StarBright Blue 700 Label Kit (#12020040) or Goat Anti-Mouse IgG SBB700 catalog antibody (#12004158) **B.** Probed with Mouse Anti-Human MBP labeled using TrailBlazer Tag Kit (#12020038) and StarBright Blue 700 Label Kit (#12020040) and Rabbit Anti-Human GAPDH (#VPA00187) detected with Goat Anti-Human StarBright Blue 520 (#12005867).

TrailBlazer Kit–Labeled Antibodies in Confocal Microscopy

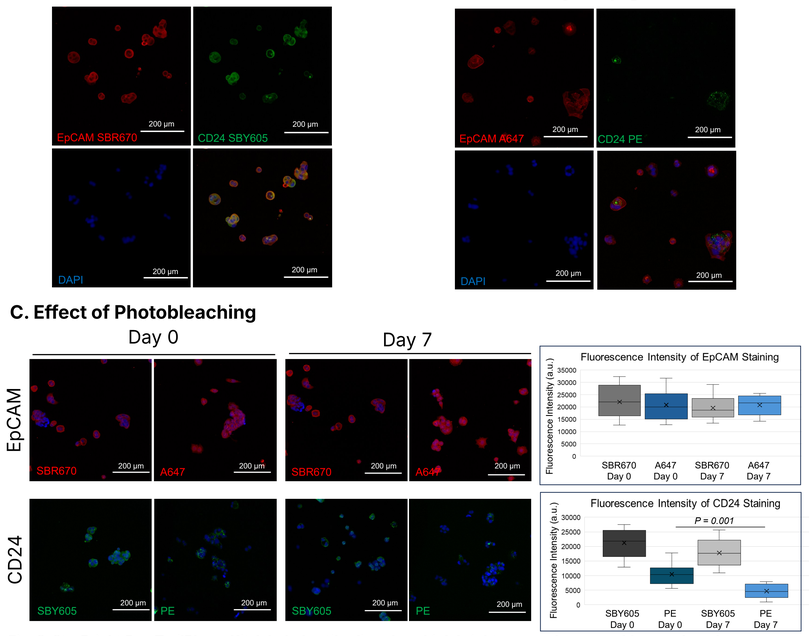

Fig. 3. StarBright Dye TrailBlazer Kit–labeled antibodies show high brightness and comparable or reduced photobleaching compared with traditional fluorophores. Confocal images of MCF7 cells stained using EpCAM (#MCA1870G) and CD24 (#MCA1379GA) labeled with the TrailBlazer Tag (#12020038) and StarBright Dye Label Kits SBR670 (#12024025) or SBY605 (#12023837) or traditional directly conjugated antibodies A647 (#MCA1870A647) and PE (#MCA1379PE). **A. and B.** Cells were imaged at Day 0 and **C**. after 7 days storage at 4°C in the dark. Fluorescence intensity quantification shows significantly reduced signal loss for PE following storage ($P = 0.001$, unpaired t-test).

TrailBlazer Kit–Labeled Antibodies in Flow Cytometry

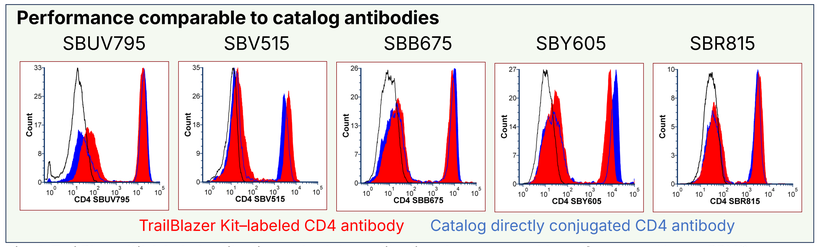

Fig. 4. TrailBlazer Kit–labeled antibodies and catalog antibodies show comparable performance. Human PBMCs were stained with Mouse Anti-Human CD4 (#MCA1267) TrailBlazer Kit–labeled antibodies (red) and catalog antibodies (blue).

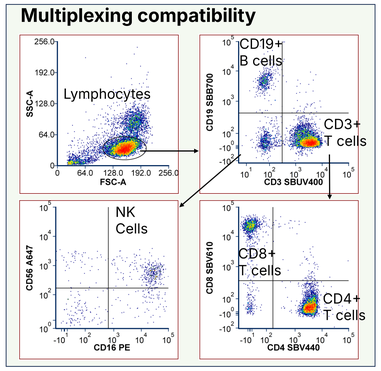

Fig. 5. Multiple TrailBlazer Kit–labeled antibodies can be used together in immunophenotyping panels. Human PBMCs were stained with TrailBlazer Kit–labeled antibodies and directly conjugated antibodies (Table 2). Cells were gated on live, single cells (not shown).

Marker	Fluorophore	Antibody Catalog #	Label Kit Catalog #
CD3	SBUV400	MCA463SBUV400	
CD4	SBV440	MCA1267	12020042
CD8	SBV610	MCA1226	12020043
CD19	SBB700	MCA1940	12020040
CD16	PE	MCA5665PE	
CD56	A647	MCA2693A647	

Table 2. Antibodies used in multiplexing panel.

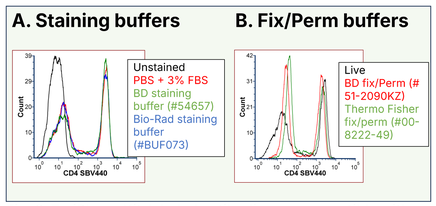

Fig. 6. TraiBlazer Kit–labeled antibodies are compatible with different buffers. Human PBMCs were stained with Mouse Anti-Human CD4 (#MCA1267) labeled using TrailBlazer Tag Kit (#12020038) and StarBright Violet 440 Label Kit (#12020042) **A.** In different staining buffers, or **B.** fixed and permeabilized using different reagents.

One Year Stability of TrailBlazer Kit–Labeled Antibodies

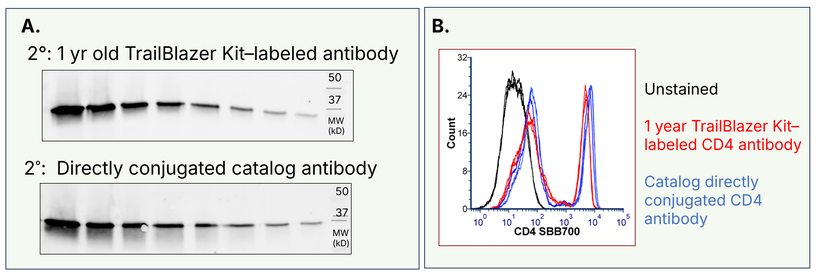

Fig. 7. High Performance of TrailBlazer Kit–labeled antibodies used after one year storage at 4°C. A. Western blot analysis of 2X serial dilution of HeLa lysate (#171YZT002). Probed with Mouse Anti-Human GAPDH (#VMA00046). Detected using Goat Anti-Mouse IgG labeled using the TrailBlazer Tag (#12020038) and StarBright Blue 700 Label Kit (#12020040) at a dilution of 1:5,000, or Goat Anti-Mouse IgG directly labeled with StarBright Blue 700 at a dilution of 1:10,000 (#12004158). **B.** Red cell lysed human peripheral blood unstained (black), stained with Mouse Anti-Human CD4 (#MCA1267) labeled using the TrailBlazer Tag Kit (#12020038) and StarBright Blue 700 Label Kit (#12020040) (red), or stained with a directly conjugated Mouse Anti-Human CD4 StarBright Dye (#MCA1267SBB700) (blue).

Conclusion

TrailBlazer Kits offer a significant advance for researchers, delivering an easy self-conjugation method resulting in stable conjugates. They enable the generation of robust, reproducible multiplexing data in complex biological studies in multiple applications.

Bulletin Number 3932 Ver A

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