Novel 355nm (and Lower) Excitable and Tuneable Emission (Blue through Red) Fluorophores Utilised in Flow Cytometry

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Labelling antibodies with a fluorescent reporter molecule allows for the detection and localisation of antigens situated within a cell, tissue or organ for wide applications in drug discovery and development. An array of fluorescent probes can be attached to different antibodies, each conveying their own distinct spectral properties that can be exploited by flow cytometry and multiphoton microscopy. The Preece group at the University of Birmingham have developed a novel class of fluorescent dyes known as triphenoxazoles. We have, to date, over 50 dyes that have an excitation profile ranging from ~250nm to ~420 nm. The dyes can be chemically tuned to emit from 400 nm through to 630 nm,[1, 2] and hence have utility to increase multiplexing in both multicolour and spectral flow cytometry.

This study focuses on antibody conjugation of one dye *via* the formation of an active ester on a novel dye (Gen-2-Dye). The reaction uses EDC/sulfo-NHS chemistry to activate the carboxylic acid functionality on the fluorophore which enables conjugation to a CD8 antibody *via* an amide linkage.

Characterisation by UV-Vis absorbance and steady-state emission spectroscopy confirmed the presence of the antibody with the distinct fluorescent signature of the Gen-2-Dye. A commercial antibody conjugation check kit also confirmed successful conjugation and antibody functionality ***(Figure 1a)***. The Gen-2-Dye•CD8 conjugate was used to stain compensation beads and displayed a bimodal distribution in the bead population relative to the unstained control ***(Figure 1b-d)***. Further optimisation is underway to increase the fluorescence intensity of the positive population of beads and extend testing to cells/tissue.

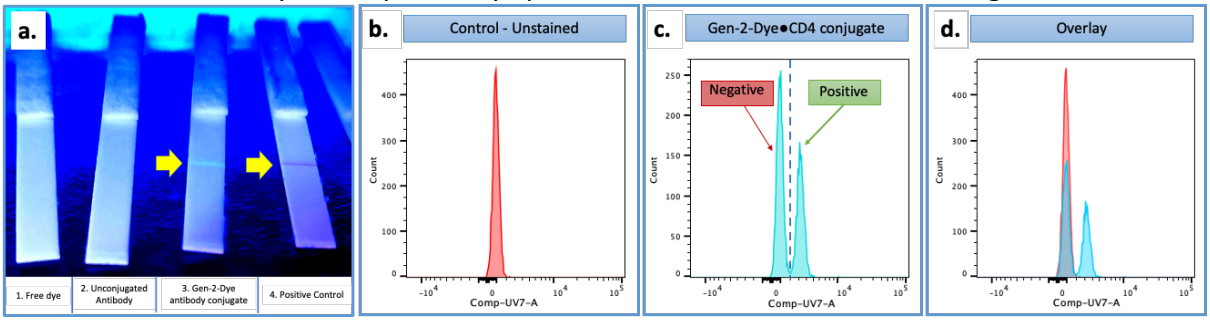

Figure 1*. **a**: conjugate check kit. **b-d**: compensation beads unstained (red), stained with Gen-2-Dye•CD4 conjugate (37 µg/mL,* 18 hr, *37 °C) [comp-UV7 channel 500-528 nm].*

References: *[1] Alex Robinson, Jon Preece, Karolis Virzbickas, Owen Jones, Dennis Zhao, Michael Butlin, Sareena Sund,* ***2019*** *U. S. Patent Application 20210070720. [2] Alex Robinson, Jon Preece, Gregory O'Callaghan, Karolis Virzbickas, Owen Jones, Dennis Zhao, Michael Butlin, Sareena Sund,* ***2019****, Patent Application 20210005824.*

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