BioTracker™ TiY Vimentin Live Cell Dye

Live Cell Dye

Cat. # SCT059

pack size: 1 mg

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

Vimentin is an intermediate filament protein which is upregulated in cells undergoing epithelial-to-mesenchymal transition (EMT), and hence is commonly used an EMT marker. Tumor initiating cells (TICs) have been implicated in clinical relapse and metastasis of a variety of epithelial cancers, including lung cancer.

BioTracker™ TiY Vimentin is a live cell fluorescent dye that selectively stains vimentin in TICs over differentiated tumor cells or normal cells. TiY can be used for visualization and enrichment of functionally active TICs from patient tumors. TiY displays better selectivity for tumorigenic cells than a CD166 antibody and can be used for TiY-staining-based cell sorting for TICs in various types of cancer (lung, CNS, melanoma, breast, renal, ovarian, colon and prostate cancer). At high concentrations, TiY shows selective anti-TIC activity over non-TIC and normal tissue cells.

Storage

Store BioTracker™ TiY Vimentin Live Cell Dye at -20°C, desiccate and protect from light

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Spectral Properties

Absorbance: 553 nm
Emission: 573 nm

Quality Control

Purity: ≥ 98% confirmed by HNMR, LC-MS and HPLC and elemental analysis.
Molar Mass: 541.87 g/mol

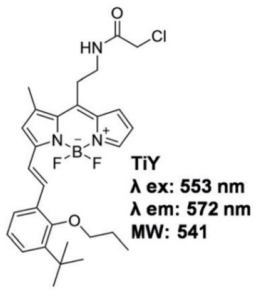

Figure 1: Chemical structure of TiY.

Protocol

Reagent Preparation for Live Cell Imaging

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Warm the vial to the room temperature and add DMSO to make a 1000X stock solution of 5-10 mM (freeze aliquots at -20°C).
3. Dilute in cell culture media at a final concentration of 1 µM and add to cells in culture. Incubate at 37°C for 60 minutes.
4. Wash cells with PBS buffer before imaging.

***Note**: Optimal concentration must be determined by end user.*

Flow Cytometric Analysis for TiY and Vimentin Antibody

1. After single cell dissociation, stain cells with TiY (1-5 µM) in suspension at 4ºC for more than 40 min.
2. Fix the cells with 4% PFA for 10 min, permeabilize in 0.2% Triton-X in DPS for 5 min and block with DPBS containing 2% BSA for 1 hour.
3. Label the cells with the antibody against vimentin-660 containing 1% BSA and 0.2% Tween-20 at 4oC overnight.
4. Wash twice between every step and measure fluorescence intensities with a FACS machine.

***Note**: Optimal concentration must be determined by end user*

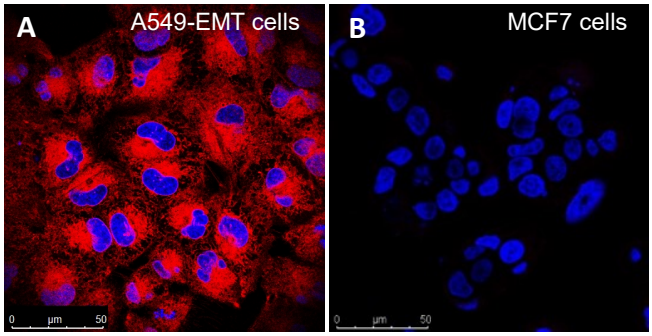

Figure 2. TiY Vimentin probe can be used to identify EMT cells. A549-EMT cells express vimentin and stain positive with TiY probe (A). MCF7 cells, epithelial cells, are negative for vimentin as shown by lack of TiY staining (B). DAPI (blue) was used to stain for cell nucleus.

References

Lee Y et al. *Identification of Tumor Initiating Cells with a Small-Molecule Fluorescent Probe by Using Vimentin as a Biomarker.* Angew Chem Int Ed Engl. 2018 Mar 5;57(11):2851-2854.

Ver.1.0/2019-07-11/SCT059MD

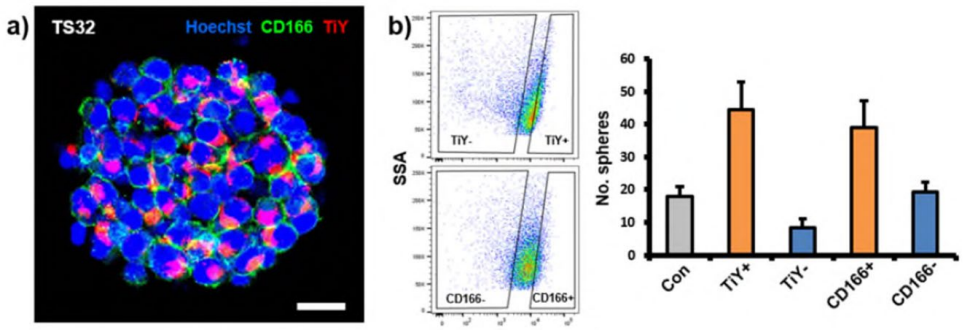

Figure 3. Superiority of TiY in TIC-specific selective staining over lung TIC marker CD166. a) Expression of CD166 at TiY-stained cells in TS32. b) Comparison of sphere forming of cells sorted by TiY or CD166 antibody dependent cell sorting from TS32 cells. Values are means ± SEM (n = 2). Scale bar = 25 μm.

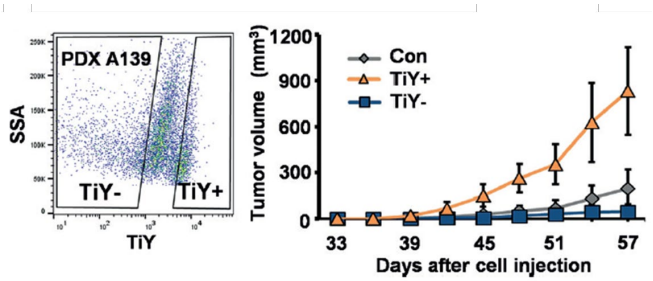

Figure 4. TiY+ cells show higher tumorigenicity. Comparison of tumorigenicity between unsorted (Con), TiY+, and TiY- cell populations in PDX 139. Values are means±SEM (n=8). The number of mice was 4 in each population. Each mouse was injected with tumor cells at 2 sites.