Data Sheet

BioTracker™ TAS2 Proteasome Activity Live Cell Probe

Live Cell Dye

SCT235

Pack Size: 500 µg

Store at -20 °C

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

The proteasome, a highly elaborate protein complex which controls protein levels and important cellular pathways, is critical to eukaryotic cell function and homeostasis. The proteasome often functions by degrading proteins that are no longer needed or damaged. The cell will selectively shuttle these proteins to be broken into smaller peptide units that can be recycled and repurposed for other essential needs of the cell. The eukaryotic 26S proteasome consists of two subcomplexes: the 19S regulatory particle (RP) and the 20S catalytic core particle (CP). The proteasome acts by tagging proteins intended for degradation with a chain of ubiquitin which is recognized by the 19S regulatory particle. After recognition, ubiquitin monomers are removed, and the remaining peptides are hydrolyzed by the 20S core particle.

Improper proteasome function has been associated with a number of diseases including a variety of cancers. This ultimately makes the proteasome of interest for detailed study and a potential therapeutic target. Although proteasome-activity based fluorescent probes have been designed previously, they suffer from poor fluorescence and selectivity challenges.

SCT235 is a substrate-based fluorescent probe intended to be cleaved and released by the eukaryotic 20S core particle subunit of the proteasome to monitor proteasome activity. TAS2 shows strong selectivity and resistance to unspecific cleavage by proteases in human serum. TAS2 can be used effectively in applications such as live-cell assays, flow cytometry, or live-cell fluorescent microscopy.

Source

SCT235 does not contain genetically modified organisms.

Quality Control Testing

Purity: ≥ 98% confirmed by HPLC. Structure confirmed by HNMR, LC-MS, and elemental analysis.

Molar Mass: 1407.12 g/mol

Storage and Handling

Store BioTracker™ TAS2 Proteasome Activity Live Cell Probe at –20 °C, desiccate and protect from light.

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

Presentation

Lyophilized. Light yellow solid.

Spectral Properties

Excitation: 496 nm

Emission: 524 nm

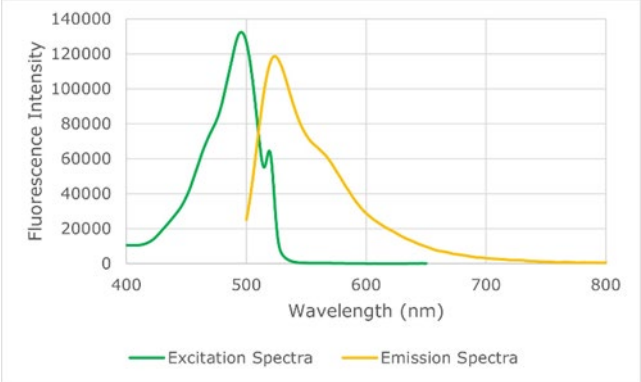

Figure 1: Probe excitation and emission data. TAS2 probe was diluted to 20 µM in 50 mM Tris HCl pH 7.5. Probe solution was then incubated with 2 µg of 20S proteasome fragment (P3988) at 37 °C for 60 minutes to induce probe activation before undergoing excitation and emission scans. Spectral scans were conducted using a PerkinElmer FL8500 Fluorescence Spectrophotometer.

Representative Data

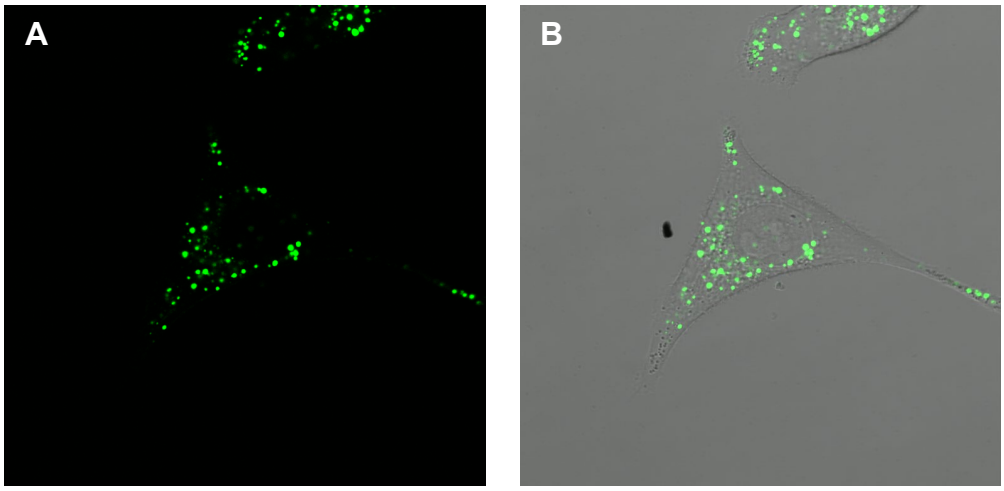

Figure 2: Confocal microscopy images of 10 µM TAS2 staining of A549 cells (**A**) merged with BrightField cell imaging (**B**). Cells were treated with TAS2 probe for 90 minutes.

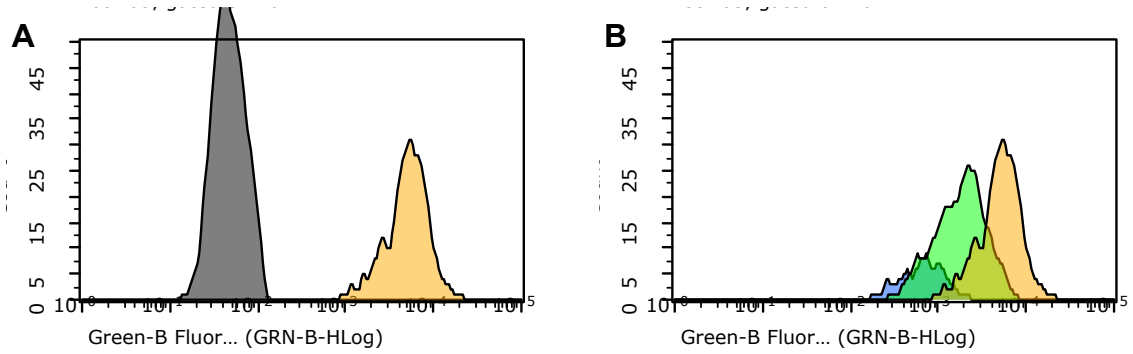

Figure 3: (**A**) Unstained A549 cells (grey) were compared to A549 cells treated with 10 µM TAS2 for 60 minutes (yellow). TAS2 probe was properly cleaved by the cellular proteasome, resulting in fluorescence. (**B**) A549 cells were pre-treated with MG-132, a proteasome inhibitor, followed by 10 µM TAS2. Uninhibited cells (yellow) were compared to cells pre-treated with 10 µM MG-132 for 30 minutes (green) and 30 µM MG-132 for 30 minutes (blue).

Protocols

Preparing TAS2 live cell probe stock solution

1. Before opening the vial, spin down the solution to bottom by a microcentrifuge or by a desktop centrifuge.
2. Warm the vial to room temperature. Prepare the TAS2 (Molecular Weight: 1407.12 g/mol) dye stock solution by dissolving the contents of one vial (500 µg) in 36 µL of DMSO to create a 10 mM stock solution.
3. Aliquot and store stock solution at –20 °C or below for longer storage.

Labeling cells

1. Culture cells in an appropriate medium and vessel for fluorescence microscopy.
2. Prepare the TAS2 staining solution by diluting the TAS2 stock solution 1:1,000 in culture medium.
3. Remove the cell culture medium from the cells.
4. Add sufficient TAS2 staining solution to cover the cells.
5. Incubate for 90 minutes, protected from light.
6. Remove staining solution and wash with PBS. Observe the cells under fluorescence microscope for green fluorescence: λ_{ex} = 495 nm, λ_{em} = 515-555 nm.

   Note: Optimal concentration must be determined by end user.

References

Zerfas BL, Coleman RW, Salazar-Chaparro AF, Macatangay NJ, Trader DJ. 2020. Fluorescent Probes with Unnatural Amino Acids to Monitor Proteasome Activity in Real-Time. ACS Chemical Biology. 15(9):2588–2596. doi:https://doi.org/10.1021/acschembio.0c00634.

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