Living Colors® HEK 293 ZsGreen Proteasome Sensor Cell Line Additional Information

(PT3769-3)

This document provides detailed technical information about a Living Colors Cell Line.

A. Monitoring the activity of the proteasome using fluorescent proteins

Protein degradation by the proteasome is at the core of many pathological processes such as inflammation, autoimmunity, neurodegenerative diseases and cancer. When developing treatments for these processes, some efforts have focused on identification of compounds that modulate the activity of the proteasome. In order to develop an assay tool to monitor proteasome activity in live cells and bypass laborious biochemical manipulations, the reef coral fluorescent protein ZsGreen (zFP506;1) was converted into a substrate for proteasomal degradation. A proteasome targeting sequence consisting of amino acids 410–461 from mouse ornithine decarboxylase (MODC) was used to direct ZsGreen to degradation by the proteasome (1,2). Data from Li and Coffino suggest that this sequence is likely to be a stronger proteasome-targeting motif than the d1 sequence described by us and others (3). The chimeric fluorescent protein ZsProSensor-1 is constitutively degraded by the proteasome in stably transfected HEK 293 cells and accumulates under conditions which alter the activity of the proteasome. This live cell assay is highly sensitive and allows quantitative monitoring of proteasome activity by microscopy, flow cytometry, and fluorometry. Using the TTP Labtech Acumen Explorer™ System, inhibition of proteasome activity by a known peptide inhibitor, ALLN, was detected at discrete time points in HEK 293 ZsGreen Proteasome Sensor Cells.

B. Reef Coral Fluorescent Proteins

ZsGreen is on of the Living Colors® Reef Coral Fluorescent Proteins, which are derived from a group of nonbioluminescent reef corals belonging to the *Anthozoa* class (4). In adapting these proteins for use as *in vivo* reporters, we have introduced a series of mutations into the corresponding full-length cDNAs to produce RCFPs with higher solubility, brighter emission, and more rapid chromophore maturation. In addition, human codon-optimized versions of each cDNA have been created for efficient translation in mammalian cells. RCFPs share structural homology to *Aequorea victoria* green fluorescent protein (GFP; 1, 5). In contrast to the color variants of *Aequorea* GFP, however, RCFPs are unique proteins encoded by distinct genes, rather than mutant variants of a single fluorescent protein. They are well tolerated by mammalian cells, and have therefore proven to be useful for creating stably transfected cell lines and transgenic organisms (5–7). For specific examples or RCFP expression and use, we suggest you search our citations database, available at **www.clontech.com**, as well as other public databases for published studies relevant to your area of interest.

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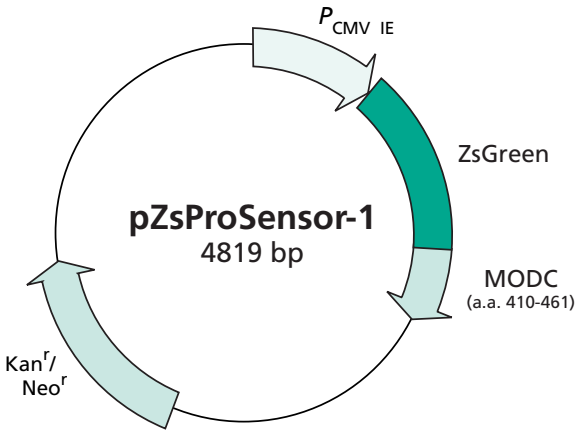

Figure 1. Targeting of ZsGreen to degradation by the proteasome. In order to convert ZsGreen wt (1) into a substrate for proteasomal degradation and use it as a sensor of proteasome activity, we generated a fusion protein between the ZsGreen fluorescent protein and amino acids 410 –461 of MODC. This vector constitutively expresses ZsProSensor-1 under the control of the CMV promoter in our stable, clonal HEK 293 ZsGreen Proteasome Sensor Cell Line.

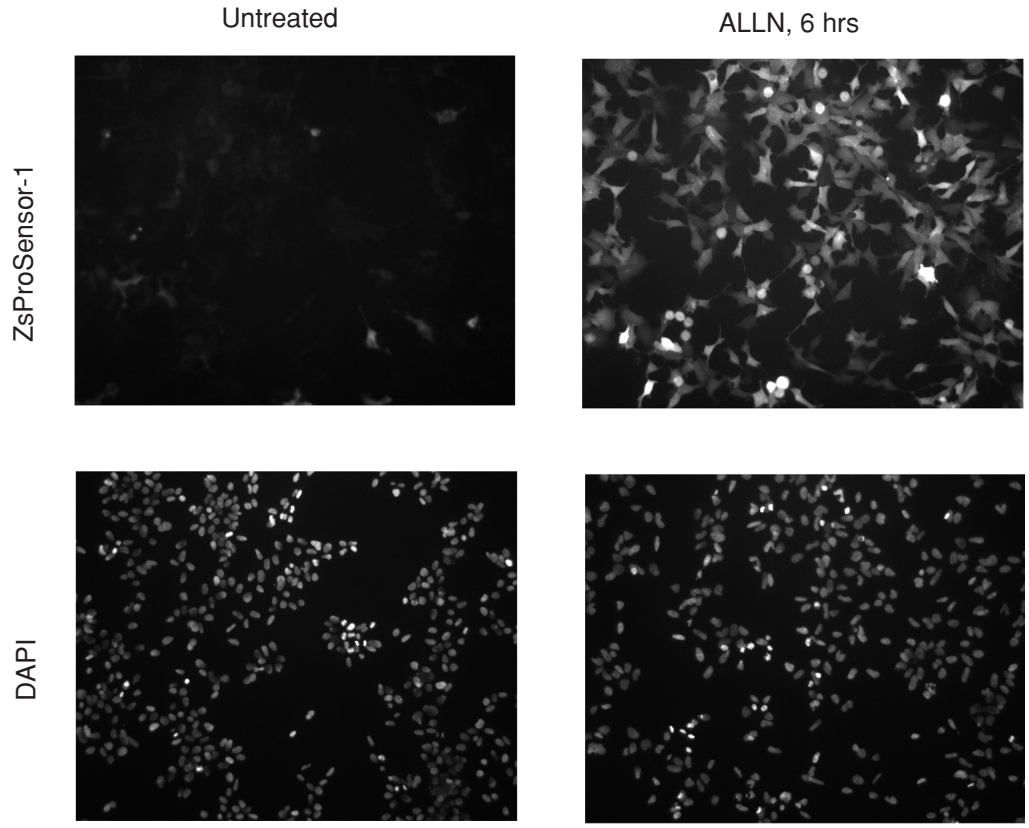

Figure 2. Sensitive microscopic detection of proteasome activity in the HEK 293 ZsGreen Proteasome Sensor Cell Line. HEK 293 ZsGreen Proteasome Sensor cells were treated for 6 hours in the presence of ALLN, a well-characterized inhibitor of proteasome-dependent proteolysis, and the green fluorescence of ZsProSensor-1 in both treated and non-treated cells was monitored by microscopy (top). A DAPI stain of cell nuclei is also shown (bottom). The analysis was performed using a Zeiss Axioskop and the micrographs were taken with the same exposure times for each fluorophore with filters identical to those used for GFP.

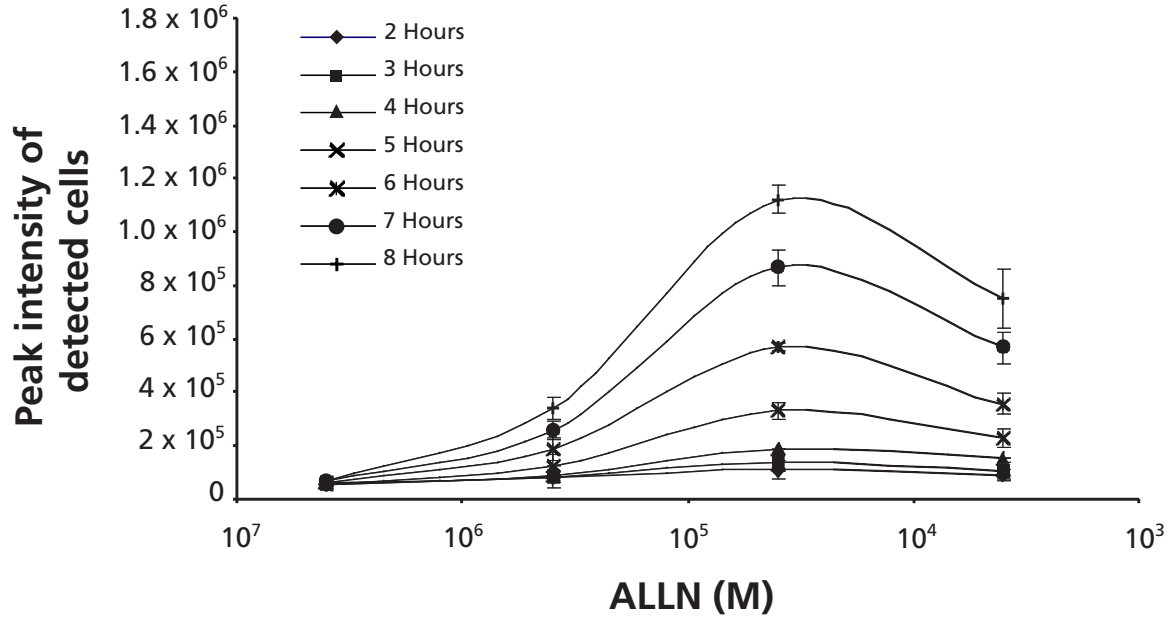

Figure 3. Detection of proteasome inhibition in HEK 293 ZsGreen Proteasome Sensor cells on the TTP Labtech Acumen Explorer™ fluorescent detection instrument. HEK 293 ZsGreen Proteasome Sensor cells were seeded at 2000 cells per well in a BD Labware 96-well glass-bottom plate. Following overnight incubation, the cells were treated with various concentrations of ALLN, a known proteasome inhibitor. The fluorescent emission of ZsProSensor-1 was measured with the green detection channel of the TTP Labtech Acumen Explorer™ fluorescent detection instrument for 2–8 hours. Whole well scans were performed with resolutions of 4 µm in the Y direction and 0.5 µm in the X direction. The throughput for this assay irrespective of plate type is approximately 15 minutes per plate. Within each well, fluorescent debris was removed and separated from single cells on the basis of their morphology values and excluded from further analysis. The number of single cells detected was reported for each inhibitor concentration and time point. The mean peak intensity values for the responding population of single cells were plotted.

References

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