

A Modulus™ Microplate Fluorometer Application Note for Testing Clontechs Living Colors® HEK 293 ZsGreen Cell Line

INTRODUCTION

The Living Colors® HEK 293 ZsGreen Cell Line from Clontech is a stably-transfected line ready for use in cell-based assay applications. ZsGreen is one of the Living Colors® Reef Coral Fluorescent Proteins derived from the *Anthozoa* class of nonbioluminescent reef corals. These proteins have been adapted for use as *in vivo* reporters by introducing a series of mutations into the corresponding full-length cDNAs. This process produces RCFPs with higher solubility, brighter emissions, and more rapid chromophore maturation.¹

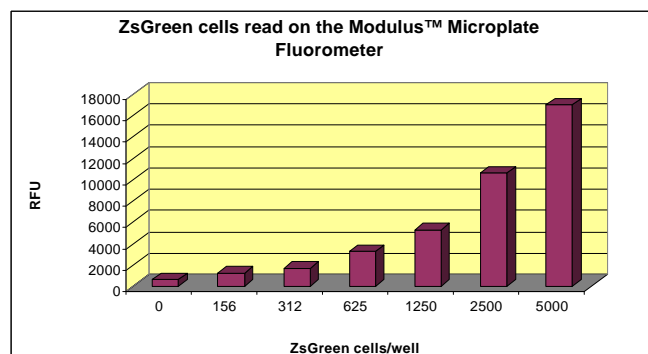
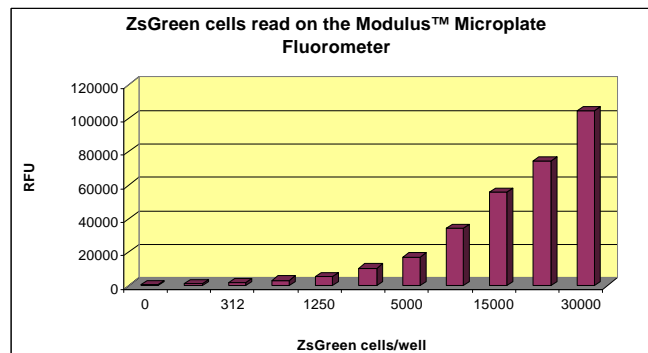
The method described in this application note uses the Turner BioSystems Modulus™ Microplate Fluorometer to successfully measure cell lines expressing fluorescent properties. This Fluorometer can detect cell numbers from as low as 156 cells per well in a 96-well microplate format.

METHOD

The following data was generated by a single instrument. ZsGreen has a maximum excitation of 496 nm and a maximum emission of 506 nm. Use the Modulus™ Microplate Fluorometer Blue Fluorescence Optical Kit which has an excitation of 490 nm and an emission range of 515 - 580 nm.

1. Count and plate cells in Costar 3603 tissue-culture treated plates with black or clean bottoms. Incubate cells overnight at 37° C with 5% CO₂. Maintain equal cell numbers per well. In this experiment, non-transfected HEK-293 cells were added to maintain a cell density of 30,000 per well.
2. The following day, cell culture media should be removed and replaced with 200 µL PBS.
3. Read the cell assay plate on the Modulus™ Microplate Fluorometer using the Fluorescence Optical Kit - Blue, 490/515-580 nm.

RESULTS



# of ZsGreen cells	Avg (n=6)	Stdv	% CV
0 (parental only)	623	8	1.3
156	1,252	88	7.0
312	1,705	130	7.6
625	3,264	331	10.1
1,250	5,301	437	8.2
2,500	10,596	500	4.7
5,000	17,001	612	3.6
10,000	34,204	1817	5.3
15,000	55,915	1472	2.6
20,000	74,435	3319	4.5
30,000	10,4472	5495	5.3

CONCLUSION

The Modulus™ Microplate Fluorometer offers both superior sensitivity and dynamic range. The Modulus™ Microplate Fluorometer achieves superior performance by use of a dedicated fluorescence detector. The detector is not shared with any other detection modes. The individual Fluorescent Optical Kit of the Modulus™ Microplate Fluorometer uses solid-state optics and a powerful wavelength-matched LED to deliver excellent sensitivity and dynamic range.

The modular approach of the Modulus™ Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or Absorbance Detection Modules as well as other accessories can be added after initial purchase.

Superior performance, ease of use, and utmost flexibility makes the Modulus™ Microplate Reader the ideal choice for today's life science laboratory.

ACKNOWLEDGEMENT

Turner BioSystems would like to express appreciation to Omar Nourzaie, Michael Haugwitz, and Jane Khau of Clontech for the assistance they provided in gathering data for this application.

REFERENCE

1. Living Colors® Volume II: Reef Coral Fluorescent Proteins.

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