

A HaloTag-based Gene Reporter System for Live-Cell Imaging and High-Throughput Screening

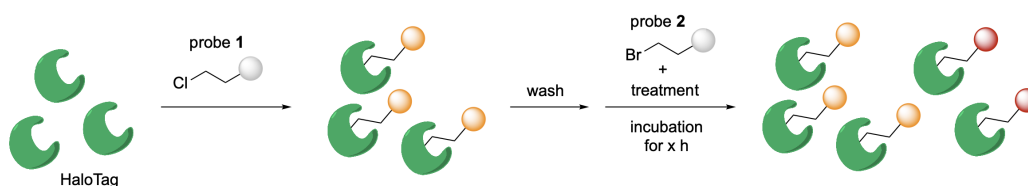
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Gene expression monitoring is a powerful tool to elucidate stress response mechanisms and dynamics. We developed a gene reporter system for BiP (binding immunoglobulin protein), a key mediator of the endoplasmic reticulum (ER) stress response.^[1]

Upon accumulation of unfolded proteins in the ER, the unfolded protein response (UPR) is activated, which consists of three pathways that are initiated by IRE1, PERK, and ATF6.^[2] The target BiP is a chaperone that assists protein folding. It is involved in the activation of the UPR as well as induced via the ATF6 pathway.^[3]

Our system is based on the co-expression of the target BiP and the self-labeling HaloTag protein^[4] in a stable cell line. The amount of HaloTag that is produced thus corresponds to the amount of BiP. By labeling HaloTag with two fluorogenic compounds with different spectral properties—a rhodamine and a silicon rhodamine—two gene expression levels can be time-stamped and visualized in a single pulse-chase experiment, providing additional information and increasing the robustness of the system. The method is suitable for live-cell fluorescence imaging as well as flow cytometry. Moreover, we are able to employ the system in high throughput screening experiments, searching for novel inducers of BiP expression.



[1] Claudio Hetz, Kezhong Zhang, Randal J. Kaufman, *Nat. Rev. Mol. Cell Biol.* **2020**, 21, 421–438.

[2] Martin Schröder, Randal J. Kaufman, *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **2005**, 569, 29–63.

[3] Matthew D. Shoulders, Lisa M. Ryno, Joseph C. Genereux, James J. Moresco, Patricia G. Tu, Chunlei Wu, John R. Yates, Andrew I. Su, Jeffery W. Kelly, R. Luke Wiseman, *Cell Reports* **2013**, 3, 1279–1292.

[4] Georgyi V. Los, Lance P. Encell, Mark G. McDougall, Danette D. Hartzell, Natasha Karassina, Chad Zimprich, Monika G. Wood, Randy Learish, Rachel F. Ohana, Marjeta Urh, Dan Simpson, Jacqui Mendez, Kris Zimmerman, Paul Otto, Gediminas Vidugiris, Ji Zhu, Aldis Darzins, Dieter H. Klauert, Robert F. Bulleit, Keith V. Wood, *ACS Chem. Biol.* **2008**, 3, 373–382.