PhotoScreen™ Luminescent Calcium Assays: Applications on the LuminoLux® and FLIPRTETRA®

Sabrina Corazza, Viniana Aguas, Silvia Boveletta, Lia Scanavacca, Giovanna Quattrocchi, Vincent Dupire, Craig Malcolm (IB Assay, Max IV, J) PerkinElmer LAS (Inc.)

Abstract
C₉²-activated photoproteins are important tools for analyzing all aspects of C₉²-mediated signal transduction processes in mammalian cells. One of their characteristics is the immediate photon release upon C₉²-biding to the constitutive photoprotein complex, which makes this system extremely useful for studying rapid receptor signal transduction of both G-protein coupled receptors involving C₉²-mediated signal transduction. Photoproteins used here were either the PhotoScreen® or iPhotina® proteins, which were reconstituted in transfected mammalian cells expressing the specific photoprotein variants. Photoproteins can be used for high-throughput calcium signaling experiments by introducing a filter system that allows for rapid determination of membrane calcium responses at high signal-to-background ratios. Data generated on the LuminoLux™ Cellular Screening Platform and FLIPRTETRA® systems with PhotoScreen® photoproteins overexpressing different classes of targets will be presented.

Background/Information
Photoprotein-mediated flash luminescence is a valuable tool for studying rapid intracellular calcium increase triggered by receptors, activators. Several different photoproteins have been used for this purpose, and we present here one of these variants for a number of photoproteins: (a) the PhotoScreen® parental cell line has been created and used for generating double-codable PhotoScreen® recombinants. These cells co-express a target gene of interest and the photoprotein for calcium detection. The first cell line, the PhotoScreen® A3 cells, was evaluated on the LuminoLux™ and FLIPRTETRA® instruments.

Methods
Cloning
Human TRPA1 (GenBank NM_000561) and human Aβ (GenBank NM_001054) cDNAs were amplified by RT-PCR from human ORL cell line. The PCR products were cloned into pCMV (Stratagene) plasmid and sequenced. The Aβ cDNA was confirmed by DNA sequencing. For the Aβ receptor, a fusion protein between γ-syn and Aβ was created in order to block the natural signaling of the Aβ receptor in the photoprotein C₉²-signaling pathway.

Cell culture
CHO-K1 cells stably expressing the C₉²-activated photoprotein (PhotoScreen®) and the Aβ gene were maintained in Dulbecco's Modified Eagles Medium (DMEM) containing 10% (v/v) fetal bovine serum. 1000 mL/1 of 1% (w/v) glucose, 10 mM of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 1x PBS and 1 mg/mL of sodium bicarbonate. 37°C and 5% CO₂ atmosphere. 100 µg/mL of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 1% (v/v) glucose, 10 mM of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 100 µg/mL of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 1% (v/v) glucose, 10 mM of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 100 µg/mL of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 100 µg/mL of L-glutamine, and 1 µg/mL of penicillin-streptomycin. 100 µg/mL of L-glutamine, and 1 µg/mL of penicillin-streptomycin.

Cell transfection and clone selection
After transfection, a selection cocktail with the Aβ-TETR-ORL cells was grown in the selective agent G418 and subclones were selected by limiting dilution. Best responding clones were selected based on the overall content of photoprotein and on the basis of the best functional response of the specific transcribed gene.

CHO PhotoScreen Aβ: Cell density optimization on the LuminoLux (suspension protocol)

CHO PhotoScreen Aβ: Pharmacology on the LuminoLux (suspension protocol)

CHO PhotoScreen Aβ: DMSO sensitivity on the LuminoLux (suspension protocol)

HEX PhotoScreen TRPA1: Clone Response Stability (suspension protocol)

HEX PhotoScreen TRPA1: Agonist pharmacology on the LuminoLux (suspension protocol)

HEX PhotoScreen TRPA1: 2' factor determination over 3 days (suspension protocol)

Concluding remarks
1. Both the CHO PhotoScreen Aβ cell line from the PhotoScreen™ Starter Kit and HEX PhotoScreen™ TRPA1 cell line display very strong stable and reproducible luminescence signal upon agonist stimulation.
2. Receptor functionality was demonstrated by monitoring flah luminescence emitted by the C₉²-activated PhotoScreen® and iPhotina® photoproteins on both FLIPRTETRA® and LuminoLux® instruments.
3. The best performing electrophysiological properties are obtained with patch clamp experiments.
4. We have determined the IC₅₀ and IC₅₀ values of different agonists and antagonists and we have obtained reproducible results, independently of the detection system applied.
5. We have assessed the assay robustness and data reproducibility by determining the Z′ values for agonists as well as antagonists.
6. We have assessed the sensitivity to DMSO of the assay to fully exclude screening processes, suitable for the identification of both GPCR and ion channels.