



Photina[®]: Q & A

1. What is Photina[®]?

Photina[®] is the new and improved **Ca²⁺ activated photoprotein** for the development of precise Ca²⁺ mobilisation assays for High-Throughput Screening (HTS).

2. What is a photoprotein?

The term **photoprotein** identifies an active complex that is capable of emitting light - **bioluminescence** - and is formed by an **apophotoprotein** - a protein not bound to the luciferin molecule - and the prosthetic group **coelenterazine** (luciferin). The binding of **Ca²⁺ ions** to the photoprotein active complex induces a conformational change that results in the oxidation of coelenterazine to coelenteramide with a subsequent emission of a **blue light flash**.

Photoproteins are present in nature in many marine organisms that emit light for a variety of purposes including breeding, feeding and defense. Apophotoproteins can be transfected into cell lines for use as **reporter genes** for detecting intracellular Ca²⁺ concentration variations.

3. What is flash luminescence?

Flash luminescence is the blue light flash produced when the active complex photoprotein is activated by Ca²⁺ ions and the prosthetic group coelenterazine is oxidized to coelenteramide. The reaction is extremely fast and the light generation lasts only for 10-20 seconds. There is a direct correlation between the intensity of light signal and the quantity of Ca²⁺ ions interacting with the photoprotein. The flash light signal can be detected by specific instrumentation.

4. Why is (flash) luminescence better than fluorescence in HTS campaigns?

Luminescence-based cellular assays offer several advantages over fluorescence-based assays in HTS campaigns:

- **Absence of background** - luminescence methods have no background compared to fluorescent dyes. This allows a higher signal-to-noise ratio for luminescence-based assays.
- **Targeting of the probe** - the expression of luminescent photoproteins can be targeted to specific subcellular compartments using appropriate targeting sequences to allow a more precise measurement of intracellular Ca²⁺ movements. The uniform distribution of fluorescent dyes into the cell cytoplasm does not allow for this degree of precision or specificity.
- **No toxicity** - luminescent apophotoproteins are transfected into the cells and are constitutively expressed without toxicity. The prosthetic group coelenterazine also is not toxic to the cells and is not subject to efflux. On the contrary, fluorescent dyes have fixed incubation times with the cells and are subject to efflux.
- **Reduced auto-fluorescence of compounds** - a consistent number of compounds tested in HTS campaigns exhibit auto-fluorescence when excited by the wavelength normally used for excitation of fluorescent dyes. These compounds show up as false positive hits. This phenomenon can be drastically reduced using luminescence as read out system.

5. Is Photina[®] superior to other photoproteins?

The light release obtained from stimulated cells expressing the photoprotein **Photina[®]** is higher compared to that obtained by other photoproteins mainly due to a slower kinetic of light release. This superior performance of Photina[®] allows the use of a reduced number of cells/well plus the use of any luminescence detector for light recording.



6. What is the average Z' factor for Photina® assays?

Several different assays have been developed in cell lines expressing the Ca²⁺ activated photoprotein **Photina**®. Assay quality has been assessed by evaluating the standard deviation and Z' factor in different plate formats (384- and 1536-MTP) on a range of luminescent readers. An average Z' factor of 0.7 was obtained for most of the assays.

7. What cell types have been tested using Photina® technology?

To date, **Photina**® has been successfully transfected and used for generation of cell-based assays in Chinese Hamster Ovary cells (CHO) and Human Embryonic Kidney (HEK293) - in both mitochondrial and cytoplasmic versions. Recently, mouse Embryonic Stem Cells expressing **Photina**® have been generated.

8. On which instruments has Photina® technology been evaluated?

Photina® performance has been evaluated with success on the following instruments:

<i>Instrument</i>	<i>MTP format</i>	<i>Manufacturer</i>
FLIPR ³	384	MDC
FLIPR ^{TETRA}	384, 1536	MDC
CyBi® Lumax	384, 1536	CyBio
FDSS6000	384	Hamamatsu
LumiLux TM	384, 1536	Perkin Elmer
MicroBeta® JET	96	Perkin Elmer
GENios TM Pro	384	Tecan
Infinite TM 200	384	Tecan

9. How many cells per well are required for Photina® assays?

The required number of cells/well varies depending on the instrument used for detecting the **Photina**® flash light signal. A suggested range for adhesion protocols is:

250-1,500 cells/well in 384-MTP
750-2,000 cells/well in 1536-MTP

10. Which targets can be addressed with Photina® technology?

Photina® technology is well suited for detecting intracellular Ca²⁺ release from internal stores mediated via **GqPCR** activation. Furthermore **Photina**® has been successfully validated for detecting Ca²⁺ influx into cells mediated by **Ca²⁺ permeable ion channels** and **Na⁺/Ca²⁺ exchangers**. In general, **Photina**® can be used as a reporter gene whenever a Ca²⁺ concentration variation takes place in a cell.

11. How does Photina® perform in HTS?

Photina® has been used in several different HTS campaigns, both in 384 as well as in 1536 MTP format. **Photina**® performance was assessed with GqPCRs as well as with ion channels permeable to Ca²⁺ and in both cases **Photina**® performance fulfilled all the criteria for a successful HTS campaign. The size of the compound libraries tested ranged from 350,000 to 1.200,000 compounds.

12. Has Photina® been tested transiently for HTS?

Cells stably expressing **Photina**® have been used for transient transfection of target genes of interest. The cell line performance varies according to the transfected gene, the expression vector used, and the transfection method. Overall the light release is diminished if compared to the signal obtained by the same target stably expressed into the **Photina**® cell line, but is still strong enough for a reliable detection.

13. Do you offer a trial period for cell lines?

Yes. We offer the opportunity to test different **Photina**® containing cell lines directly in our laboratories at Axxam using the instrumentation present on site - FLIPR^{TETRA}, CyBi® Lumax, and CCD camera-based Lumibox. You will be assisted during the trial period by an Axxam scientist. Alternative arrangements can be made for those unable to travel to Axxam in Milan. Please **contact us** for more information on evaluating **Photina**®.

14. What are the licensing terms for Photina® technology?

Please **contact us** to discuss licensing of **Photina**® Technology.