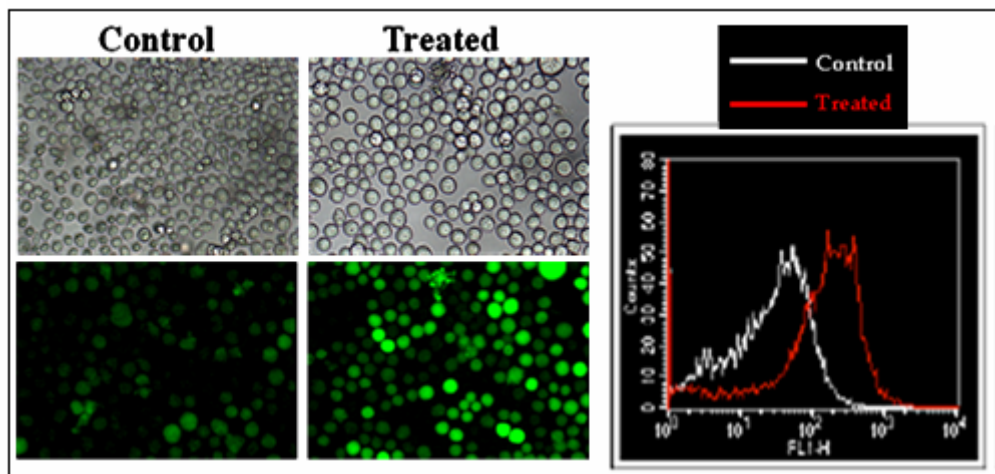


Cellular biosensors for the identification of fetal hemoglobin inducers

Prof. Roberto Gambari - University of Ferrara

The University of Ferrara developed a cellular model to quickly screen a variety of potential therapeutic compounds for the transcriptional induction of fetal haemoglobin (HbF), preferentially acting on the human γ -globin gene promoter, aimed to treat β thalassaemias

Microscope and FACS fluorescence analysis after treating the biosensor with a HbF inducer.



1. Description of the product

The cellular biosensor consists of recombinant cellular clones (K562 cells), in which the pCCL.Prom β .HcRed1.Prom γ .EGFP vector was stably introduced. This vector contains a green (EGFP) and a red (HcRed1) fluorescence protein genes under the control of γ -globin and β -globin promoters, respectively.

In these systems, the fluorescence signal is strictly connected with the transcriptional regulation of globin promoters: in particular, increase of green EGFP signal is consistent with a γ -globin gene promoter driven activity, whereas increase of the far red HcRed1 signal is associated with β -globin promoter activity. The fluorescence analysis can be both qualitative, through the use of a fluorescence inverted microscope, and quantitative, by using instruments like FACS (Fluorescence-activated cell sorting).

2. Innovative aspects of the product

Pharmacologically mediated stimulation of human γ -globin gene expression and increase of HbF levels is considered a potential therapeutic modality in haematological disorders, including β -thalassaemia and sickle cell anaemia. Based on this assumption, there is a great interest to identify and characterize HbF inducers but since now the systems available are time-consuming, laborious, expensive and give few indications on the

mechanism of action of the inducer molecules. The most innovative aspect of the biosensor developed is the combination of a simple and cheap assay with a short DNA construct (it makes it possible to use different cell lines for transfection) and with the possibility of automation, obtaining a reliable method for a rapid and preventive screening of possible inducers of fetal haemoglobin.

3. Main advantages of the offer

The cellular biosensor developed allows a simple and rapid assay to determine the transcriptional effect of high numbers of molecules on β or γ globin gene promoters, resulting in an increase of red or green fluorescence signals, respectively.

The system can be easily automated: for instance, the fluorescence detection can be performed in a 96-well plate format, allowing the contemporary analysis of 96 samples and requiring very small volumes. Moreover, it is possible to conduct single live cell time lapse imaging studies under perfectly controlled environmental conditions.

Finally, this system is very versatile. First of all, it can be used in the pharmaceutical field for the screening of molecules aimed to the therapy of other diseases: it is only necessary to identify the correct promoter to put upstream the fluorescent protein gene and then the transcriptional effect of possible drugs can be evaluated. Second, several different applications are possible in other fields, for example food, agricultural or environmental: in all cases only the construct to introduce in cells has to be changed appropriately.

4. Technology keywords

Fluorescence, drugs, high-throughput screening, β thalassemia, HbF inducer.

5. Current stage of development

Developed and tested in laboratory.

6. Intellectual property rights

No patent on this product. Several patents on HbF inducers.

Technical and scientific publications

Cellular biosensors for the identification of fetal hemoglobin inducers. Breveglieri G, Salvatori F, Finotti A, Bertuzzi I, Destro F, Falzoni S, Bianchi N, Borgatti M, Zuccato C, Feriotto G, Breda L, Rivella S, Gambari R. *Minerva Biotecnologica*, 2007; 19 (04), 123.

CONTACT

info@biopharmanet.eu

Tel.: +39 0521 905073 - Fax: +39 0521 905006