University of Ferrara

UNIFE PATENTS

Biomedical devices

RATIOMETRIC PROBE FOR MEASURING EXTRACELLULAR ATP

DNA | Extracellular ATP | IRES sequence | Firefly luciferase

OBIETTIVI PER LO SVILUPPO SOSTENIBILE

3 SALUTE E BENESSERE

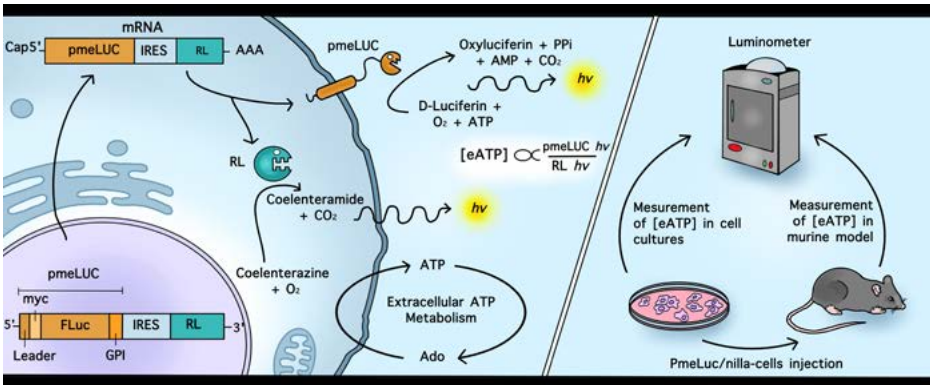
TECHNOLOGY AREA
Healthcare, biomedical

RELEVANT MARKETS
Biotechnology, hospital

PATENT OWNER: University of Ferrara, University of Padua
GEOGRAPHICAL EXTENSION: PCT
PRIORITY NUMBER: 102023000022473
FILING DATE: 26/10/2023
PATENT STATUS: filed
AVAILABILITY: licensable

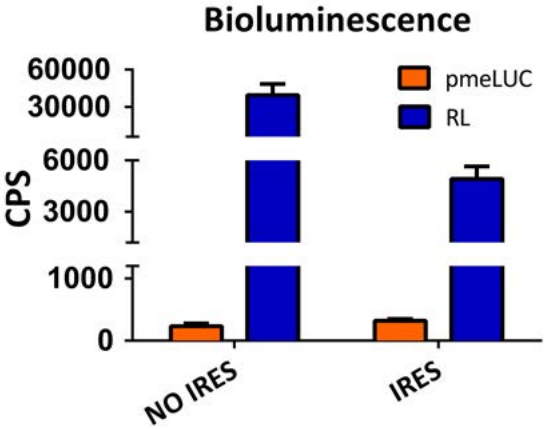
BRIEF DESCRIPTION OF THE INVENTION

The invention relates to a DNA construct for measuring extracellular ATP levels and consists of a bicistronic plasmid vector with two recombinant genes separated by an IRES sequence.



CHARACTERISTICS OF THE INVENTION

The probe object of the invention is useful for measuring the concentration of extracellular ATP in living organisms. In particular, the invention relates to a DNA construct comprising or consisting of the following elements: a DNA sequence coding for an extracellular ATP dependent luciferase and anchored to the plasma membrane; an IRES sequence; a DNA sequence coding for a cytoplasmic ATP independent luciferase. The pmeLUC/nilla makes it possible to use the membrane luciferase pmeLUC to quantify extracellular ATP, which in turn allows the measurement of ATP changes near the cell membrane, both in vivo and in vitro. Thanks to the introduction of Renilla luciferase, the new pmeLUC/ nilla probe overcomes the main shortcoming for the in vivo use of pmeLUC, i.e. the dependence of the intensity of the luminescence signal not only on the actual concentration of extracellular ATP but also on the expression level of the probe, which can be variable in different tissues. The use of Renilla luciferase in the new pmeLUC/nilla construct, on the other hand, makes it possible to normalise the pmeLUC signal, thus obtaining a numerical value dependent solely on ATP concentration.



POSSIBLE APPLICATIONS

The invention offers the possibility of obtaining a normalised measurement of extracellular ATP both in vivo and in vitro. It can therefore be used in the study of ATP as a signal molecule in physiological and pathological processes.

The invention can be used for research and development of drugs that act on purinergic receptors or, more generally, modulate inflammatory processes.

ADVANTAGES

- **The pmeLUC/nilla allows the membrane luciferase pmeLUC to be used to quantify extracellular ATP, making it possible to measure the sudden changes in ATP near the cell membrane.**
- **The IRES sequence allows simultaneous translation of pmeLUC and Renilla, which depend on the same promoter for expression. It also allows for less of the latter protein (Renilla) than the former (pmeLUC), facilitating normalisation.**
- **The invention is ready to be commercialised, with no further development required.**

Università degli Studi di Ferrara

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e Rapporti con il territorio

Via Saragat,1 Corpo B - II piano
44122 Ferrara
Tel. 0532/293202
e-mail: utt@unife.it

www.unife.it