High-throughput quantification of ochronotic pigment formation in Escherichia coli to evaluate the potency of human 4-hydroxyphenylpyruvate dioxygenase inhibitors

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Organisation
Name of the organisation: Vrije Universiteit Brussel (VUB)
Department: Pharmaceutical and Pharmacological Sciences
Specific Research Group or Service: In Vitro Toxicology and Dermato-Cosmetology
Country: Belgium
Geographical Area: Brussels Region

Partners and collaborations
RWTH Aachen

SCOPE OF THE METHOD

| The Method relates to | Human health |
|-----------------------|-------------|
| The Method is situated in | Basic Research |
| Type of method | In vitro - Ex vivo |
DESCRIPTION

Method keywords

high throughput
bacterial cells
colorimetric
4-hydroxyphenylpyruvate dioxygenase Inhibitor
Screening assay

Scientific area keywords

biotechnology
in vitro
Cell culture
microbiology

Method description

This method is a straightforward, colorimetric, and inexpensive high-throughput screening system in bacteria which depends on the activity of human HPD. This screening assay is based on the formation and accumulation of a melanin-like ochronotic pigment which has a characteristic brown color. In the presence of an HPD-inhibitor this ochronosis process will be reduced or even prevented when the HPD activity is blocked by a human HPD inhibitor. The screening system will allow to identify new and human-specific HPD inhibitors and evaluate their therapeutic potential for the development of therapies for tyrosine-dependent inborn errors of metabolism.

Lab equipment

- Biosafety Cabinet;
- 96-multiwell plates (flat bottom, V-bottom);
- LB medium;
- Multichannel pipettes;
- Shaker incubator;
- Erlenmeyer culture flasks.

Method status
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages
- Reliable and robust ($Z' = 0.87$);
- Specific and sensitive readout in short measurement time;
- Cost-effective.

Modifications
Method can be miniaturised to 384 and 1536-well format using adjusted equipment including liquid-handling robotics.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References
Neuckermans, J., Mertens, A., De Win, D. et al. A robust bacterial assay for high-throughput screening of human 4-hydroxyphenylpyruvate dioxygenase inhibitors. Sci Rep 9, 14145 (2019).

Associated documents
scientific reports.pdf

Links
A robust bacterial assay for high-throughput screening of human 4-hydroxyphenyl...