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

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Geographical Area Brussels Region
Name of the organisation RWTH Aachen
Department Institute of Biotechnology
Country Germany

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 equipement 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…

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