

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

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
This method makes use of	Other (e.g. bacteria): <i>Escherichia coli</i>

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...](#)

PARTNERS AND COLLABORATIONS

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