# 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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**Department** Pharmaceutical and Pharmacological Sciences

**Specific Research Group or Service** In Vitro Toxicology and Dermato-Cosmetology

**Country** Belgium

**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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