

High-throughput quantification of ochronotic pigment formation in Escherichia coli to evaluate the potency of human 4-hydroxyphenylpyruvate dioxygenase inhibitors *Created on: 25-05-2020 - Last modified on: 28-02-2022* 

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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 highthroughput 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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