	EPP-SOP-ITB02
ITB, Stuttgart	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Expression of recombinant oleate hydratase from Elizabethkingia meningoseptica

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experimenter 1	Rebecca Demming		10th April 2018

Instruction

Expression of recombinant oleate hydratase from Elizabethkingia meningoseptica (Em-OAH).

1 Introduction / Purpose

The purpose of this protocol is to express recombinant Em-OAH in *E.coli.*

2 Equipment and chemicals

2.1 Equipment

Baffled flasks
Centrifuge

2.2 Chemicals

Kanamycin
Isopropyl β-D-1-thiogalactopyranoside (IPTG)

2.3 Bacterial strains

E. coli BL21 (DE3)

2.4 Other materials

Plasmid pET28a(+)::Em-OAH

3 Media and buffers

3.1 TB medium

12 g/L tryptone (1.2%)
24 g/L yeast extract (2.4%)
4 g/L glycerol (0.4%)
ad dd H20 900ml

Adjust pH with 100ml sterile 10x TB-buffer after autoclaving

3.2 TE Buffer (10X)

KH_2PO_4 0.17M
K_2HPO_4 0.72M

4 Procedures

400 ml TB medium in a 2 L baffled flask with 30 µg mL^{-1} final kanamycin concentration was inoculated with an overnight preculture of E. coli BL21 (DE3) pET28a(+)::Em-OAH to an OD 600 of 0.02.

Cultures were incubated at 37 °C and 180 rpm and gene expression was induced at an OD 600 of 0.5-0.7 by addition of 0.5 mM finale IPTG concentration. After 20 hours of incubation at 20 °C and 140 rpm, cells were harvested by centrifugation (12 min, 8000 x g, 4 °C) and washed twice in 50 mL citrate buffer (50 mM, pH 6). Pellets were stored at -20 °C until usage.

5 remarks/troubleshooting

6 Biosafety

No biosafety issues were associated with this protocol when applied to E. coli. The protocol was developed and performed at an S1 laboratory.

7 Acknowledgements

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