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HMA determination

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This protocol is used to determine the yield of HMA produced by *E. coli* by using HPLC.

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HPLC (Agilent 1260)
Welch Xtimate C8 column (4.6 × 250 mm, 3.5 μm)
KH₂PO₄
100% acetonitril
ZY
50×M
50×5052
1000×Trace element
1M MgSO₄·7H₂O
antibiotic
20% arabinose
5 × M9
1 M CaCl₂
20% glucose
ddH₂O
0.22μm membrane filter
HPLC sample bottle
Centrifuge

Wash the sample bottle with ddH₂O for 3 times and absolute ethanol for 1 time by using ultrasonic cleaner.


Prepare 8 mM KH₂PO₄ (pH 2.4) and filter it before use.

Activation of the cells

- 1 Inoculate 5 ml of LB medium with 1% volume of E. coli culture and culture at 37°C for 12h.
 **200 rpm, 37°C, 12:00:00**

Antibiotics of corresponding resistance should be added into the LB medium.

Induction of protein expression

- 2 Prepare ZYM-5052 medium (5ml) by mix ingredients below: (for detailed recipe
 **ZYM-5052.docx**

A	B
ZY	4800μl
50×M	100μl
50×5052	100μl
1000×Trace element	5μl/10μl
1M MgSO ₄ ·7H ₂ O	5μl/10μl
antibiotic	5μl
20% arabinose	50μl

- 3 Inoculate 5 ml of ZYM-5052 medium with 1% volume of E. coli LB culture and culture at 37°C for 12h. 🔄 **200 rpm, 37°C, 12:00:00**

Fermentation

- 4 Prepare M9 medium (10ml) by mix ingredients below:

A	B
5 × M9	2ml
1M MgSO ₄ ·7H ₂ O	20μl
1 M CaCl ₂	1μl
20% glucose	200μl
ddH ₂ O	7.8ml

- 5 Centrifuge 60D bacteria in 1.5 ml eppendorf (EP) tube at 4,200 x rpm for 10 minutes at room temperature.
- 6 Decant or aspirate and discard the culture media.
- 7 Use 200μl M9 medium to spirate and dis completely resuspend cell pellet and culture at 37°C for 12h. 🔄 **200 rpm, 37°C, 12:00:00**

Sample preparation

- 8 Centrifuge at 15,000 x g for 10 minute at room temperature.

- 9 Take 100 µl of supernatant into another EP tube and mix it with 900µl ddH₂O.
- 10 Filter the sample with 0.22µm membrane filters and inject the sample into a clean sample bottle.

HPLC (high performance liquid chromatography)

- 11 HMA was measured on an HPLC (Agilent 1260) equipped with a UV detector and a Welch Xtimate C8 column (4.6 × 250 mm, 3.5 µm). The samples were analyzed under the following gradient of eluent A (8 mM KH₂PO₄, pH 2.4) and eluent B (100% acetonitril): 5 min 10% B, 20 min linear gradient to 30% B, 3 min linear gradient to 50% B, 5 min 50% B, 2 min linear gradient to 10% B, 5 min 10% B. HMA was detected at 215 nm.