



# HMA determination

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Oct 07, 2021

1 *Works for me*

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## ABSTRACT

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

## DOI

[dx.doi.org/10.17504/protocols.io.byuupwww](https://dx.doi.org/10.17504/protocols.io.byuupwww)

## PROTOCOL CITATION

Shuning Guo 2021. HMA determination. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.byuupwww>



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## CREATED

Oct 07, 2021

## LAST MODIFIED

Oct 07, 2021

## PROTOCOL INTEGER ID

53876

## MATERIALS TEXT

HPLC (Agilent 1260)

Welch Xtimate C8 column (4.6 × 250 mm, 3.5 μm)

KH<sub>2</sub>PO<sub>4</sub>

100% acetonitril

ZY

50×M

50×5052

1000×Trace element

1M MgSO<sub>4</sub>·7H<sub>2</sub>O

antibiotic

20% arabinose

5 × M9

1 M CaCl<sub>2</sub>

20% glucose

ddH<sub>2</sub>O

0.22μm membrane filter

HPLC sample bottle

Centrifuge

### BEFORE STARTING

Wash the sample bottle with ddH<sub>2</sub>O for 3 times and absolute ethanol for 1 time by using ultrasonic cleaner.

Prepare 8 mM KH<sub>2</sub>PO<sub>4</sub> (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 <sub>4</sub> ·7H <sub>2</sub> 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 <sub>4</sub> ·7H <sub>2</sub> O	20μl
1 M CaCl <sub>2</sub>	1μl
20% glucose	200μl
ddH <sub>2</sub> O	7.8ml

- 5 Centrifuge 6OD 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 ddH<sub>2</sub>O.
- 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 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 KH<sub>2</sub>PO<sub>4</sub>, 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.