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## Creation of low-oxygen conditions. V.2

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**Protocol status:** Working

**We use this protocol and it's working**

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### Abstract

We use Na<sub>2</sub>SO<sub>3</sub> to create low-oxygen conditions in our experiment.



## Materials

LB medium


Na<sub>2</sub>SO<sub>3</sub>

IPTG

SMART SENSOR AR8010+ Dissolved Oxygen Meter

Tecan Spark<sup>®</sup> multimode microplate reader

## Safety warnings

 Please wear gloves for the experiment.

## Changes of dissolved oxygen with time in the LB medium with different concentrations of Na<sub>2</sub>SO<sub>3</sub>.

- 1 Prepare LB medium with different concentrations of Na<sub>2</sub>SO<sub>3</sub>.

1.1

Na <sub>2</sub> SO <sub>3</sub> (100g/L)	LB medium
0μL	20mL
20μL	20mL
40μL	20mL
100μL	20mL
200μL	20mL
400μL	20mL

- 2 Use Dissolved Oxygen Meter to measure dissolved oxygen of LB medium with different concentrations of Na<sub>2</sub>SO<sub>3</sub> in 0h、1h、2h、5h.

## β-Gal enzyme activity determination—Changes of ABS with time in the LB medium with different concentrations of Na<sub>2</sub>SO<sub>3</sub>.

- 3 Add 50ml LB medium and 200ul bacteria solution to conical flask, shake overnight at 37°C.
- 4 Take 5 ml in 5 50ml centrifuge tubes separately, centrifuge the bacteria at 3000xg at room temperate for 5 min.Discard the supernatant.
- 5 Prepare LB medium with different concentrations of Na<sub>2</sub>SO<sub>3</sub>.  
dd Na<sub>2</sub>SO<sub>3</sub> to LB medium.

Na <sub>2</sub> SO <sub>3</sub> (100g/L)	LB medium
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00g/ L)	
0ul	20ml
100ul	20ml
200ul	20ml
400ul	20ml
800ul	20ml

- 6 Add LB medium with different concentrations of  $\text{Na}_2\text{SO}_3$  to 5 50ml centrifuge tubes separately.
- 7 Add 2ul IPTG to 5 50ml centrifuge tubes separately, and resuspend. Shake 5h at 37°C.
- 8 Use Microplate Reader to measure ABS with different concentrations of  $\text{Na}_2\text{SO}_3$  in 0 h, 1h, 2h, 5h.