# Creation of low-oxygen conditions. V.2

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1 Works for me

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ABSTRACT

We use $Na_2SO_3$ to create low-oxygen conditions in our experiment.

MATERIALS TEXT

LB medium
$Na_2SO_3$
IPTG
SMART SENSOR AR8010+ Dissolved Oxygen Meter
Tecan Spark® 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 $Na_2SO_3$.

1 Prepare LB medium with different concentrations of $Na_2SO_3$.

1.1

| Na2SO3(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 $Na_2SO_3$ in 0h、1h、2h、5h.

## β-Gal enzyme activity determination—Changes of ABS with time in the LB medium with different concentrations of Na2SO3.

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 $Na_2SO_3$.
dd $Na_2SO_3$ to LB medium.

| Na2SO3(100g/L) | LB medium |
|---|---|
| 0ul | 20ml |
| 100ul | 20ml |
| 200ul | 20ml |
| 400ul | 20ml |
| 800ul | 20ml |

6 Add LB medium with different concentrations of $Na_2SO_3$ to 5 50ml centrifuge tubes separately.

7 Add 2ul IPTG to 5 50ml centrifuge tubes separately,and reascend.  Shake 5h at 37°C.

8 Use Microplate Reader to measure ABS with different concentrations of $Na_2SO_3$ in 0 h、1h、2h、5h.