

myo-Inositol Colorimetric Microplate Assay Kit

Cat #: orb1473556 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

Detection and Quantification of myo-Inositol Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

INTRODUCTION

myo-Inositol is a cyclitol present in most eukaryotic cells and exists as the predominant isomer of 1,2,3,4,5,6-cyclohexanehexol. As a key component of eukaryotic cell signalling, myo-inositol functions as crucial second messengers in the form of inositol (poly)phosphates and phosphatidylinositides. The abundance of myo-inositol in nature makes it an essential compound for plants and animals, and many microorganisms are equipped with catabolic pathways to enable the utilisation of myo-inositol as a sole carbon source.

myo-Inositol Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of myo-Inositol in a variety of samples. myo-Inositol is oxidised by NAD⁺ in the presence of myo-Inositol dehydrogenase. myo-Inositol is measured by the increase in absorbance at 492 nm.

KIT COMPONENTS

| Component | Volume | Storage |
|-----------------------|------------|----------------------|
| 96-Well Microplate | 1 plate | |
| Assay Buffer I | 20 ml x 1 | 4 °C |
| Assay Buffer II | 20 ml x 1 | 4 °C |
| Reaction Buffer | 10 ml x 1 | 4 °C |
| Substrate | Powder x 1 | -20 °C |
| Diluent | 2 ml x 1 | 4 °C |
| Coenzyme | Powder x 1 | -20 °C, keep in dark |
| Enzyme | Powder x 1 | -20 °C |
| Dye Reagent A | Powder x 1 | 4 °C, keep in dark |
| Dye Reagent B | 1 ml x 1 | 4 °C, keep in dark |
| Standard | Powder x 1 | 4 °C |
| Plate Adhesive Strips | 3 Strips | |

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| Manual | 1 Manual | |
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MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 492 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

REAGENT PREPARATION

Substrate: Briefly centrifuge prior to opening. Add 1 ml distilled water, shock to dissolve before use. Store at -20 °C for 1 month.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent, shock to dissolve before use. Store at -20 °C for 1 month.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Store at -80 °C for 1 month.

Dye Reagent A: Add 5 ml distilled water to dissolve before use. Store at -20 °C for 1 month.

Standard: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, mix, the concentration will be 20 mmol/L. Store at -20 °C for 1 month. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625/0.312 mmol/L.

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 800 µl distilled water for 5×10^6 cells or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 100 µl Assay Buffer I mix, and 100 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 800 µl distilled water, transfer it into the centrifuge tube; then add 100 µl Assay Buffer I mix, and 100 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

If the sample does not contain any protein or less protein, it can be assayed directly. If the sample contains more protein, the samples should be cleared by mixing 800 µl sample with 100 µl Assay Buffer I and 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a clean tube for detection (dilution factor $n = 1.25$).

Note: If assay buffers I and II were used for sample deproteinization, **Blank** should be prepared parallelly by mixing 800 µl distilled water with 100 µl Assay Buffer I and 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm, take the supernatant as the control.

ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent* | Sample** | Standard | Blank |
|--|----------|----------|--------|
| Reaction Buffer | 100 µl | 100 µl | 100 µl |
| Sample | 10 µl | -- | -- |
| Standard | -- | 10 µl | -- |
| Distilled water | -- | -- | 10 µl |
| Substrate | 10 µl | 10 µl | 10 µl |
| Coenzyme | 10 µl | 10 µl | 10 µl |
| Enzyme | 10 µl | 10 µl | 10 µl |
| Mix, incubate at 37 °C for 15 minutes. | | | |
| Dye Reagent A | 50 µl | 50 µl | 50 µl |
| Dye Reagent B | 10 µl | 10 µl | 10 µl |
| Mix, cover the plate adhesive strip, incubate at 37 °C for 15 minutes, record absorbance measured at 492 nm. | | | |

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment testing several doses to ensure the readings are within the standard curve range.

CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n \text{ (mmol/L)}$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1. According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} \times n \text{ (}\mu\text{mol/mL)}$$

2.2 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (W \times V_{\text{Sample}} / V_{\text{Assay}})} \text{ (}\mu\text{mol/g)}$$

2.3 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times (N \times V_{\text{Sample}} / V_{\text{Assay}})} \text{ (}\mu\text{mol}/10^4)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of sample, g

N: the quantity of cell or bacteria, $N \times 10^4$

V_{Standard}: the volume of standard in assay procedure, mL

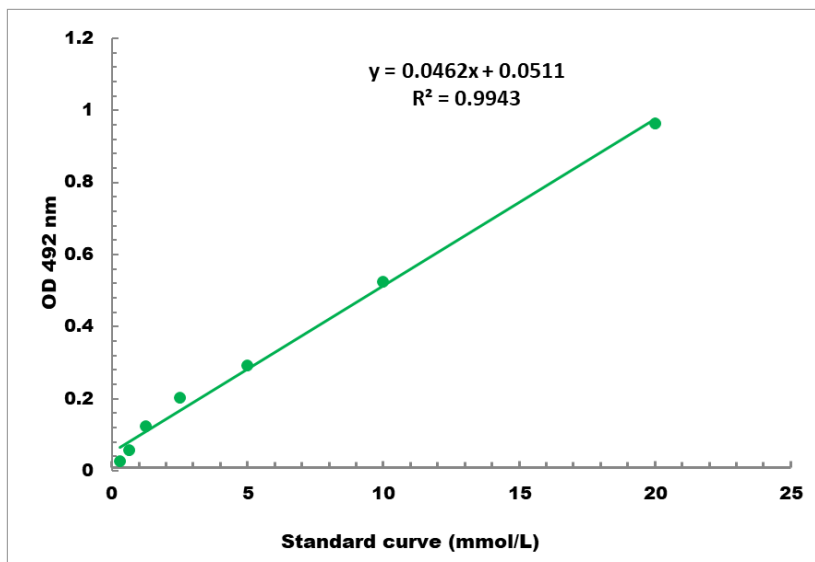
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of distilled water, Assay Buffer I and Assay Buffer II, mL

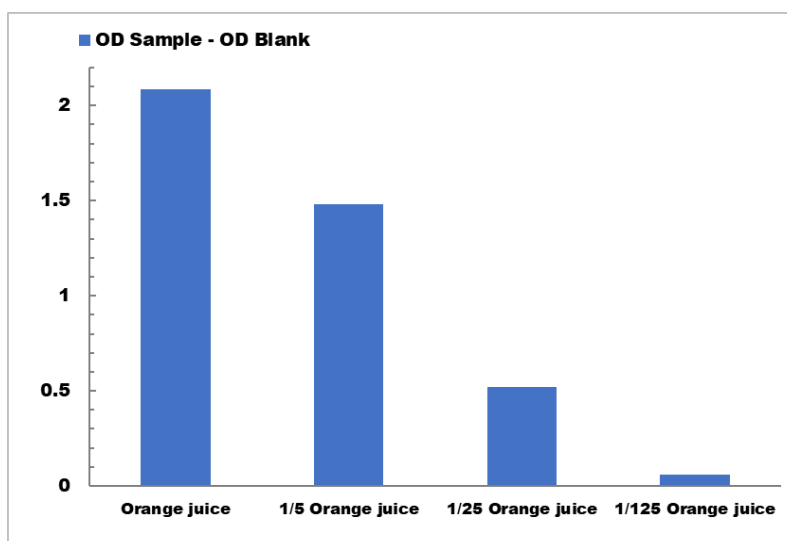
C_{Standard}: the standard concentration, $\mu\text{mol/mL}$

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.2 mmol/L - 20 mmol/L



Determination of myo-Inositol in orange juices.