myo-Inositol

ColorimetricMicroplate Assay Kit

User Manual

Catalog # CAK1265

(Version 1.7D)

Detection and Quantification ofmyo-InositolContentin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. 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-InositolColorimetricMicroplate 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-Inositolis measured by the increase in absorbance at 492 nm.

II.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	Powderx 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	
Technical Manual	1 Manual	

III. 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

IV. REAGENTPREPARATION

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

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

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

Dye Reagent A: Add 5 mldistilled 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 standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625/0.312mmol/L.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 800 µl distilled waterfor 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 mixagain, centrifuged at 10,000 rpm for 10minutes, 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 mixagain, centrifuged at 10,000 rpm for 10minutes, 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 µlsample with 100 µlAssay Buffer I and 100 µl Assay Buffer II.Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a cleantubefor detection (dilution factor n = 1.25).

Note: If assay bufferI and II were used for sample deproteinization, **Blank**should be prepared parallelly by mixing 800 µldistilled water with100 µl Assay Buffer Iand 100 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm,take the supernatant as the control.

VI. ASSAY PROCEDURE

Add following reagents intothe 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	50µl	50µl	50µl
Dye Reagent B	10µl	10µl	10µl
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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

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

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

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

2.2. According to the weight of sample

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

2.3.According to the quantity of cells or bacteria

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

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 ×10^4

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

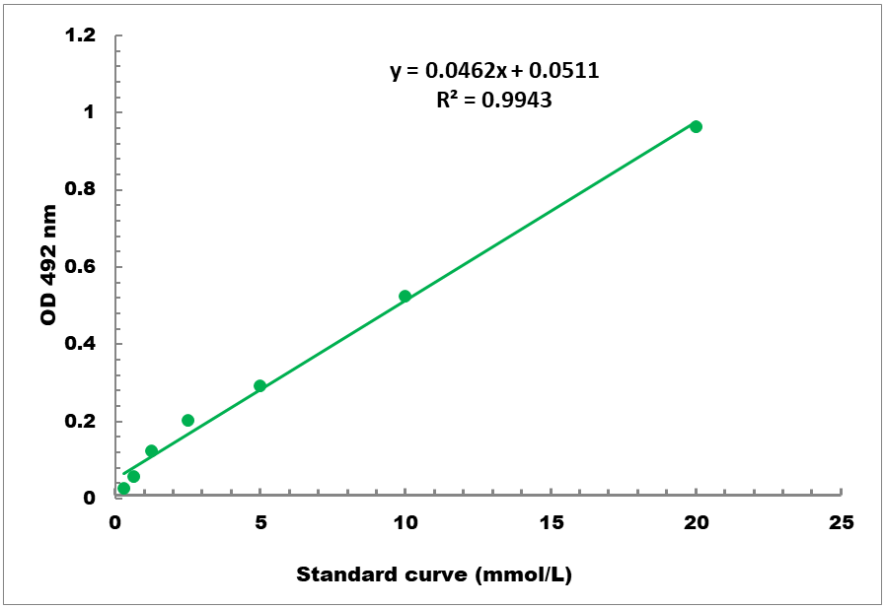
V_{Sample}: the volume of sample in assay procedure, mL

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

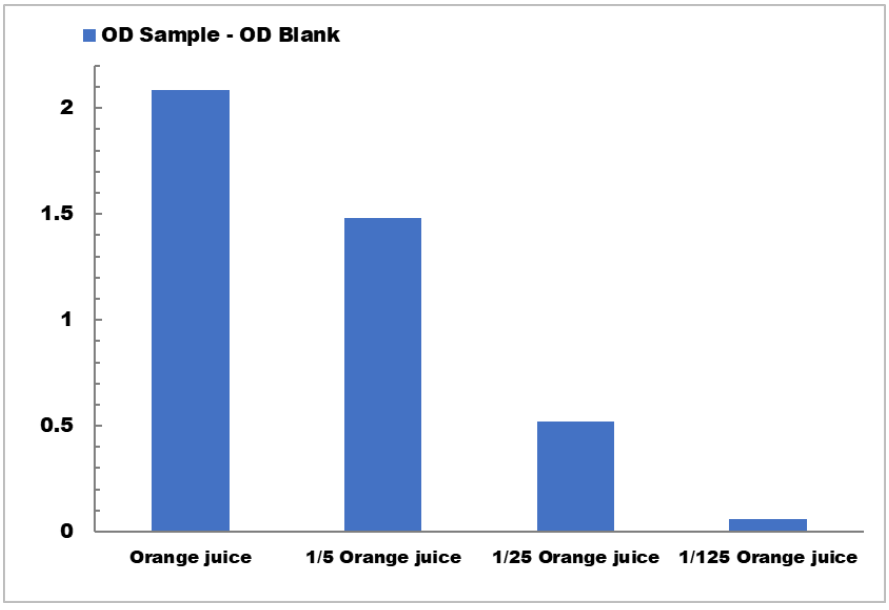
$C_{Standard}$: the standard concentration, μmol/mL

VIII. 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