myo-InositolMicroplate Assay Kit

User Manual

Catalog # CAK1265

(Version 1.2A)

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

II. 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

III.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 1	4 °C
Assay Buffer II	30 ml x 1	4 °C
Reaction Buffer I	10 ml x 1	4 °C
Reaction Buffer II	10 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Enzyme I	Powder x 1	-20 °C
Enzyme II	0.1 ml x 1	4 °C
Dye Reagent	Powderx 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Coenzyme: add 1 mlReaction Buffer II to dissolve before use.

Enzyme I: add 1 mlReaction Buffer I to dissolve before use.

Enzyme II: add 1 mlReaction BufferII to dilute before use.

Dye Reagent: add 5 mldistilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, mix, the concentration will be 20 mmol/L.

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 500 µl distilled waterfor 5×10^6 cells or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); then add 250 µl Assay Buffer I mix, and 250 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 0.5ml distilled water, transfer it into the centrifuge tube;then add 250 µl Assay Buffer I mix, and 250 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 proteins, it can be assayed directly.

If the sample contains proteins,the samples should be cleared by mixing 500 µlsample with 250 µlAssay Buffer I and 250 µl Assay Buffer II.Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a cleantubefor detection (dilution factor n = 2).

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer I	50 µl	50 µl	50 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme I	10 µl	10 µl	10 µl
Mix, incubate at 37 °C for 15 minutes.			
Reaction Buffer II	60 µl	60 µl	60 µl
Coenzyme	10 µl	10 µl	10 µl
Enzyme II	10 µl	10 µl	10 µl
Dye Reagent	50µl	50µl	50µl
Mix, cover the plate adhesive strip,incubate at 37 °C for 10 minutes, measured at 492 nm and recordthe absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the quantity of cells or bacteria

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

$$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

2. According to the weight of sample

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

$$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

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

$$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times n$$

V_{Sample}: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Assay}: the volume of distilled water, Assay Buffer I and Assay Buffer II, 1 ml;

$C_{Standard}$: the standard concentration, 20mmol/L = 20μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

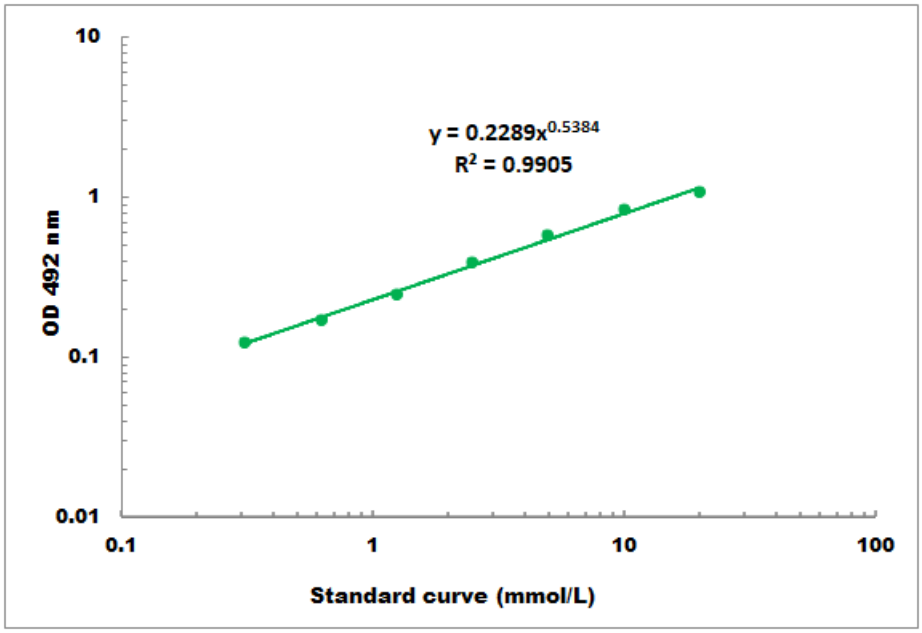
W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

n: dilution factor.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES