myo-Inositol Assay Kit

ARG83432 myo-Inositol Assay Kit can be used to measure myo-inositol in urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83432

Package: 96 wells

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INTRODUCTION

myo-Inositol plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells, the various inositol phosphates. In addition, inositol serves as an important component of the structural lipids phosphatidylinositol (PI) and its various phosphates, the phosphatidylinositol phosphate (PIP) lipids.

PRINCIPLE OF THE ASSAY

The myo-Inositol Assay Kit can measure myo-Inositol in urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids. This kit based on oxidised of myo-Inositol by NAD+ in the presence of myo-Inositol dehydrogenase. The increase in absorbance at 492 nm is directly proportional to the concentration of myo-Inositol.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Enzyme I , Enzyme II and Coenzyme at -20°C, all other components store at 4°C.

Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	RT
Standard	1 vial (lyophilized)	4°C
Reaction Buffer I	10 ml	4°C
Reaction Buffer II	10 ml	4°C
Coenzyme	1 vial (lyophilized)	-20°C
Enzyme I	1 vial (lyophilized)	-20°C
Enzyme II	1 vial (lyophilized)	-20°C
Assay Buffer I	30 ml	4°C
Assay Buffer II	30 ml	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Plate sealer	3 adhesive strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 492 nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Enzyme I, Enzyme II and Coenzyme at -20°C, all other components store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue samples- Weigh 0.1 g tissue, homogenize with 0.5 ml distilled water, transfer it into the centrifuge tube; add 250 µl Assay Buffer I mix, and 250 µl Assay Buffer II mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection.

Cell and Bacteria samples- Collect cell / bacteria into centrifuge tube, discard the supernatant after centrifugation, add 500 µl distilled water for 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 mix again, centrifuged at 10,000 rpm for 10 minutes, take the supernatant into a new centrifuge tube for detection

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 µl sample with 250 µl Assay Buffer I and 250 µl Assay Buffer II. Centrifuge 10 min at 10,000 rpm. Transfer the supernatant into a clean tube for detection (dilution factor n = 2).

REAGENT PREPARATION

- Enzyme I: Reconstitute the Enzyme I with 1 ml of Reaction Buffer I. Allow the Enzyme I keep on bench for few minutes. Make sure the Enzyme I is dissolved completely.
- Enzyme II: Reconstitute the Enzyme II with 1 ml of Reaction Buffer II. Allow the Enzyme II keep on bench for few minutes. Make sure the Enzyme II is dissolved completely.
- Coenzyme: Reconstitute the Coenzyme with 1 ml of Reaction Buffer II. Allow the Coenzyme keep on bench for few minutes. Make sure the Coenzyme is dissolved completely.
- Reagent Dye: Reconstitute the Reagent Dye with 5 ml of Distilled Water. Allow the Reagent Dye keep on bench for few minutes. Make sure the Reagent Dye is dissolved completely.
- Standards: Reconstitute the Standards with 1 ml of Distilled water, the concentration will be 20 µmol / mL. Allow the Standards keep on bench for few minutes. Perform 2-fold serial dilution of the top standards to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Mix equal Assay Buffer I and Assay Buffer II (ex. 250 µl +250 µl) as control.

1. Add **50 µl** of **Reaction Buffer I** into All wells.
2. Add **10 µl** of **Sample**, **Standard**, **Control** and **Distilled water** into appropriate wells in the plate.
3. Add **10 µl** of **Enzyme I** to each All wells.
4. Mix well. Incubate at **37°C** for **15 min**.
5. Add **60 µl** of **Reaction Buffer II** into each All wells.
6. Add **10 µl Coenzyme** into each All wells.
7. Add **10 µl Enzyme II** into each All wells.
8. Add **50 µl Reagent Dye** into each All wells.
9. Mix well. Incubate at **37°C** for **15 min**. Read the OD at **492nm**.

Summary of myo-Inositol Assay Procedure

Reagent	Sample	Standard	Control	Blank
Reaction Buffer I	50 µl	50 µl	50 µl	50 µl
Sample	10 µl	-	-	-
Standard	-	10 µl	-	-
Control	-	-	10 µl	-
Distilled water	-	-	-	10 µl
Enzyme I	10 µl	10 µl	10 µl	10 µl
Mix well. Incubate at **37°C** for **15 min**				
Reaction Buffer II	60 µl	60 µl	60 µl	60 µl
Coenzyme	10 µl	10 µl	10 µl	10 µl
Enzyme II	10 µl	10 µl	10 µl	10 µl
Reagent Dye	50 µl	50 µl	50 µl	50 µl
Mix well. Incubate at **37°C** for **15 min**. Read the OD at **492nm**				

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 20 µmol /mL;

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

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 mL;

$V_{standard}$: the volume of standard, 10 µl = 0.01 mL;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 mL.

W: the weight of sample, g;

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

B. Formula:

a). According to the weight of sample

myo-Inositol (µmol/g) =

$[(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } (W \text{ X } V_{Sample} / V_{assay})]$

$=20 \text{ X } (OD_{Sample} - OD_{blank}) / [(OD_{Standard} - OD_{Blank}) \text{ X } W]$

b). According to the volume of sample

myo-Inositol (µmol/ml) = $[(C_{Standard} \text{ X } V_{standard}) \text{ X } (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \text{ X } V_{Sample})]$

$=20 \text{ X } (OD_{Sample} - OD_{blank}) / (OD_{Standard} - OD_{Blank})$

c). According to the cell or bacteria

myo-Inositol (μmol/10^4 cell) = [($C_{Standard}$ X $V_{standard}$) X (OD_{Sample} – OD_{blank})] / [($OD_{Standard}$- OD_{Blank}) X (V_{Sample} X N / V_{assay})]

=20 X (OD_{Sample}- OD_{blank}) / [($OD_{Standard}$- OD_{Blank}) X N]

3. Detection range:

The detection range is from 0.2 μmol/ml- 20 μmol/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.