Selenium Microplate Assay Kit

User Manual

Catalog # CAK1209

(Version 1.6E)

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

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

I. INTRODUCTION

Selenium naturally appears in water and some foods. While people only need a very small amount, selenium plays a key role in the metabolism. Selenium has attracted attention because of its antioxidant properties. Antioxidants protect cells from damage. Evidence that selenium supplements may reduce the odds of prostate cancer has been mixed, but most studies suggest there is no real benefit. Selenium does not seem to affect the risk of colorectal or lung cancer.

Selenium Microplate Assay Kit is designed to measure selenium directly in biological samples without any pretreatment. Selenium catalyzes the oxidation of phenylhydrazine to azo ion by potassium chlorate. The intensity of the color, measured at 520nm, is directly proportional to the selenium concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 1	4 °C
Assay Buffer II	Powder x 1	4 °C
Reaction Buffer I	5 ml x 1	4 °C
Reaction Buffer II	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard (0.3 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Assay Buffer II: add 30 ml distilled water to dissolve before use.

Reaction Buffer II: add 5 ml distilled water to dissolve before use.

Dye Reagent: add 9 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

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 water for 5×10^6 cell 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.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 0.5 ml distilled water, transfer it into the centrifuge tube; 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.

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 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).

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Reaction Buffer I	50 µl	50 µl	50 µl
Reaction Buffer II	50 µl	50 µl	50 µl
Dye Reagent	90 µl	90 µl	90 µl
Mix, cover the plate adhesive strips, put the plate into the convection oven, 90 °C for 20 minutes. When cold, record absorbance measured at 520 nm.			

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

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

$$= 0.3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

2. According to the weight of sample

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

$$= 0.3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

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

$$= 0.3 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times n$$

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, 0.3 mmol/L = 0.3 μmol/ml;

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

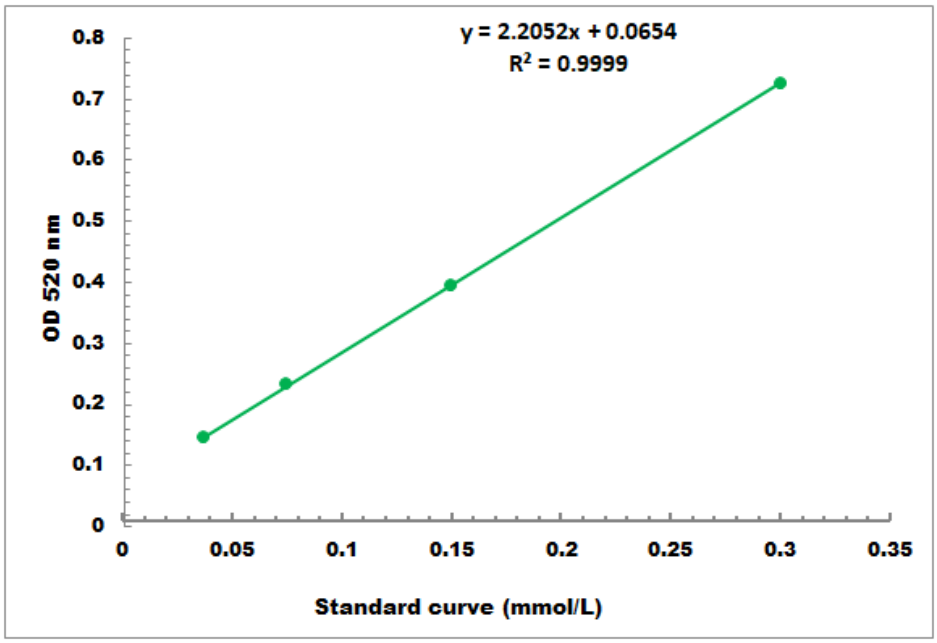
W: the weight of sample, g;

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: 0.03 mmol/L - 0.3 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES