Selenium Assay Kit

ARG83405 Selenium Assay Kit can be used to measure Selenium in urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83405

Package: 96 wells

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INTRODUCTION

Selenium, which is nutritionally essential for humans, is a constituent of more than two dozen selenoproteins that play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection.

PRINCIPLE OF THE ASSAY

Selenium Assay Kit determined Selenium based on the catalyzes the oxidation of phenylhydrazine to azo ion by potassium chlorate. The increase in absorbance at 520 nm is directly proportional to the Selenium concentration.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (30 mmol/ml)	1 ml	4°C
Assay Buffer I	30 ml	4°C
Assay Buffer II	30 ml	4°C
Reaction Buffer I	5 ml	4°C
Reaction Buffer II	1 vial (lyophilized)	4°C
Reaction Dye	1 vial (lyophilized)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 520 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all component 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.

For tissue- 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.

For cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 500 µl distilled water for 5 × 106 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.

For liquid- 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).

Note: If the sample does not contain any proteins, it can be assayed directly.

REAGENT PREPARATION

- **Reaction Buffer II:** Reconstitute the Substrate with **5 ml** of **distilled water**. Allow the Reaction Buffer II on bench for few minutes. Make sure the Reaction Buffer II is dissolved completely and mixed thoroughly before use.
- **Reagent Dye:** Reconstitute the Reagent Dye with **9 ml** of **distilled water**. Allow the Reagent Dye keep on bench for few minutes. Make sure the Reagent Dye is dissolved completely and mixed thoroughly before use.
- **Standard:** 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.

1. Sample wells: Add **10 µl Sample** into Sample wells.
2. Standard wells: Add **10 µl** of **Standard Buffer** into Standard wells.
3. Add **50 µl** of **Reaction Buffer I** into each wells.
4. Add **50 µl** of **Reaction Buffer II** into each wells.
5. Add **90 µl** of **Reagent Dye** into each wells.
6. Mix well. Incubate at **90°C** for **10 min**.
7. Mix well. Read the OD at **520 nm**.

Summary of Selenium Assay Kit Procedure

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
Reagent Dye	90 µl	90 µl	90 µl
Substrate	30 µl	-	-
Mix well. Incubate at **90°C** for **10 min**			
Read the OD at **405 nm**.			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples and control.

2. Calculation:

A. Definition:

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

$C_{Standard}$: the concentration of Standard, 0.3 μmol/ml;

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

$V_{Standard}$: the volume of reaction Standard, 10 μl = 0.01 ml;

W: the weight of sample, g;

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

n: dilution factor

B. Formula:

a). According to the volume of sample

Se (μmol/ml) =

$n \times (OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

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

b). According to the weight of sample

Se (μmol/g) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W)]$

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

c). According to the cell or bacteria

Se (μmol /10^4) =

(OD_{Sample} - OD_{Blank}) X ($C_{Standard}$ X $V_{Standard}$) / [($OD_{Standard}$ - OD_{Blank}) X (V_{Sample} X N)]

= 0.3 X (OD_{Sample} – OD_{Blank}) / [($OD_{Standard}$ – OD_{Blank}) X N]