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

Store all components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (300 nmol/mL)	1 ml	4°C
Assay Buffer A	30 ml	4°C
Assay Buffer B	1 vial (lyophilized)	4°C (protect from light)
Reaction Buffer A	5 ml	4°C
Reaction Buffer B	1 vial (lyophilized)	4°C
Reagent Dye	1 vial (lyophilized)	4°C (protect from light)
Plate sealer	3 adhesive strips	

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 A mix, and 250 µl Assay Buffer B 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 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 250 µl Assay Buffer A mix, and 250 µl Assay Buffer B 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 A and 250 µl Assay Buffer B. 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

- **Assay Buffer B:** Reconstitute the **Assay Buffer B** with **30 ml** of **distilled water**. Allow the **Assay Buffer B** on bench for few minutes. Make sure the Assay Buffer B is dissolved completely and mixed thoroughly before use. Keep in dark and store at 4 °C for 6 months after reconstitution.
- **Reaction Buffer B:** Reconstitute the **Reaction Buffer B** with **5 ml** of **distilled water**. Allow the **Reaction Buffer B** on bench for few minutes. Make sure the Reaction Buffer B is dissolved completely and mixed thoroughly before use. Store at 4 °C for 6 months after reconstitution.
- **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. Keep in dark for immediate use or store at -20 °C for 3 days after reconstitution.
- **Standard:**

Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 300 µmol/L, 150 µmol/L, 75 µmol/L, 37.5 µmol/L.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Add following reagents into the microplate:

1. Sample wells: Add **10 µl** of **Sample** into Sample well.
2. Standard wells: Add **10 µl** of **Standard Buffer** into Standard well.
3. Blank wells: Add **10 µl** of **Distilled water** into Blank well.
4. Add **50 µl** of **Reaction Buffer A** into each wells.
5. Add **50 µl** of **Reaction Buffer B** into each wells.
6. Add **90 µl** of **Reagent Dye** into each wells.
7. Mix, cover the plate adhesive strips, put the plate into the convection oven, Incubate at **90°C** for **20 min**.
8. When cold, 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 A	50 µl	50 µl	50 µl
Reaction Buffer B	50 µl	50 µl	50 µl
Reagent Dye	90 µl	90 µl	90 µl
Mix, cover the plate adhesive strips, put the plate into the convection oven, Incubate at 90°C for 20 min.			
When cold, read the OD at 520 nm.			

Note:

1. Reagents must be added sequentially and should not be premixed prior to addition.
2. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
3. 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.

CALCULATION OF RESULTS

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

2. Calculation:

A. Definition:

Slope: the absorbance slope of standard curve

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

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

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

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

V_{assay}: the volume of Assay Buffer A and Assay Buffer B in sample preparation, mL

W: the weight of total sample, g;

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

n: the dilution factor

B. Formula:

1. According to the slope of the standard curve

Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

Se (mmol/L) =

$[((OD_{Sample} - OD_{Blank})$- Intercept) / (Slope)] x n

2.According to one point of the standard OD and concentration

a). According to the protein concentration of sample

Se (U/mg) =

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

b). According to the weight of sample

Se (μmol/g) =

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

c). According to the volume of sample

Se (μmol/ml) =

$\{[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (V \times V_{Sample})/(V + V_{Assay})]\}$

d). According to the cell or bacteria

Se (μmol/10^4) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times N / V_{assay})]$