Selenium Assay Kit

Catalog No.: abx298910

Size: 100 Assays

Storage: Store all kit components at 4°C.

Application: For quantitative detection of Selenium activity in serum, plasma, tissue homogenates cell lysates, cell culture supernatants and other biological fluids.

Detection Range: 0.03 mmol/L – 0.3 mmol/L

Introduction: Selenium (Se) is a group VI element with similar bonding structures to sulphur and oxygen. In biology it is most commonly found as a selenocysteine residue; a rare non-standard amino acid occasionally placed into a protein when the ribosome reads a UGA stop codon close to a Selenocysteine Insertion Sequence (SECIS). The selenium atom allows the protein to carry out certain reduction reactions; enzymes that use this include glutathione peroxidase and thioredoxin reductase. Excessive intake of selenium salts causes garlic odour on the breath, loss of hair and nails, neurological damage, and liver cirrhosis; deficiency causes a susceptibility to some diseases, hypothyroidism, and recurrent miscarriages.

Abbexa's Selenium Assay Kit is a quick, convenient, and sensitive method for measuring and calculating selenium content. Selenium reacts with the reagents in this kit to produce an absorbance at 520 nm. The intensity of the color is proportional to the concentration of selenium, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer 1: 30 ml
3. Assay Buffer 2: 30 ml
4. Reaction Buffer 1: 5 ml
5. Reaction Buffer 2: 1 vial
6. Dye Reagent: 1 vial
7. Standard (0.3 mmol/L): 1 ml

Materials Required But Not Provided

1. Microplate reader (520 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Sonicator
7. Mortar
8. Convection oven

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Reaction Buffer 2 Solution**
  Add 5 ml of distilled water into the Reaction Buffer 2 vial and mix thoroughly to prepare the Reaction Buffer 2 Solution. Ensure that the Reaction Buffer 2 has completely dissolved prior to use.

- **Dye Reagent Solution**
  Add 9 ml of distilled water into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

2. Samples

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 500 µl of distilled water. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Add 250 µl of Assay Buffer 1 and mix thoroughly. Add 250 µl of Assay Buffer 2 and mix thoroughly. Centrifuge at 10,000 rpm at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 500 µl of distilled water. Add the homogenate to a centrifuge tube, add 250 µl of Assay Buffer 1 and mix thoroughly. Add 250 µl of Assay Buffer 2 and mix thoroughly. Centrifuge at 10,000 rpm at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

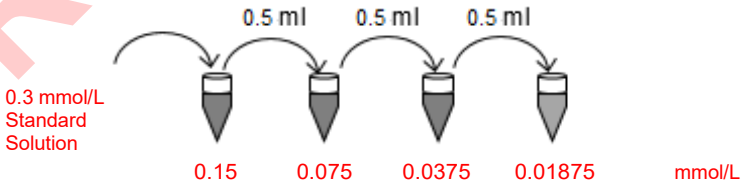
- **Liquid samples**
  Liquid samples can be used directly if they do not contain any proteins. If the samples contain proteins, take 500 µl of sample and add 250 µl of Assay Buffer 1 and 250 µl of Assay Buffer 2, then mix thoroughly. Centrifuge at 10,000 rpm at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. (Note: dilution factor is 2 if Assay Buffer 1 and 2 are added to the sample.)

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentrations within the detection range of the kit.

1. Label 4 tubes with 0.15 mmol/L, 0.075 mmol/L, 0.0375 mmol/L, and 0.01875 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 0.3 mmol/L Standard Solution to the 1st tube, and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96-well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of prepared standards to the standard wells.
5. Add 10 µl of distilled water to the blank wells.
6. Add 50 µl of Reaction Buffer 1 to each well.
7. Add 50 µl of Reaction Buffer 2 to each well.
8. Add 90 µl of Dye Reagent Solution to each well.
9. Tap the plate gently to mix. Incubate at 90°C for 10 minutes in a convection oven.
10. Allow the plate to cool to room temperature, then read and record absorbance at 520 nm.

C. Calculations

Selenium concentration per g of sample:

$$\mathbf{Se\ (\mu mol/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.3}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Selenium concentration per 10^4 cells or bacteria:

$$\mathbf{Se\ (\mu mol/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.3}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Selenium concentration per ml of sample:

$$\mathbf{Se\ (\mu mol/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times f}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{0.3 \times f} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (0.3 mmol/L = 0.3 µmol/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
f	Dilution factor
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.01 ml)