（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K776-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader(415-425 nm)

Detection range：0.06-16.00 mmol/L

Elabscience® Selenium Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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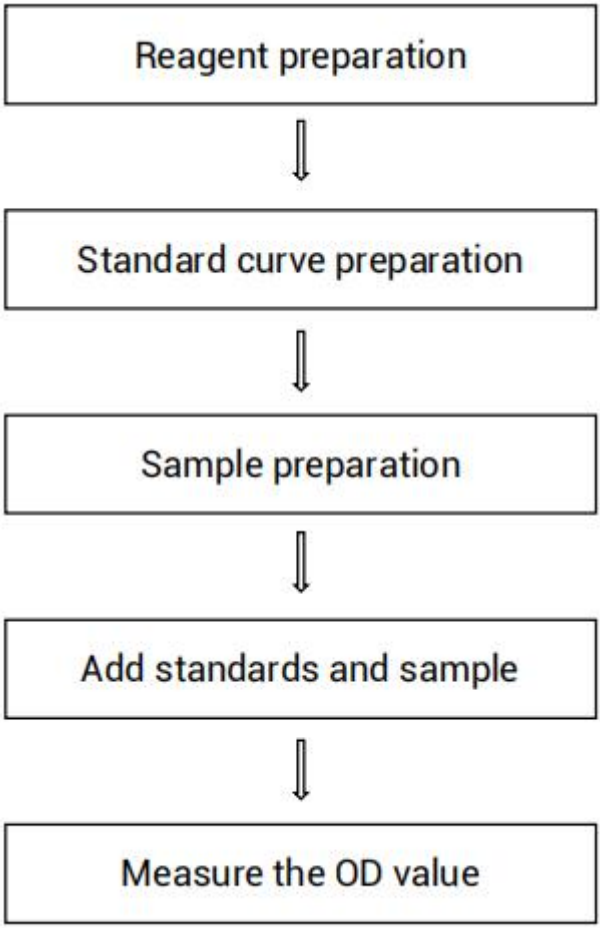
Assay summary



Intended use

This kit can be used to measure selenium content in animal tissue and cell samples.

Detection principle

Selenium is an essential microelements in the human body. It participates in many metabolic pathways of the human body, and has the functions of delaying aging, enhancing immunity and preventing many diseases. The intake of the selenium through daily diet. Excessive intake of selenium can cause selenium poisoning, leading to multiple organ damage, and insufficient intake can also cause a variety of diseases, such as Kashin-beck disease, etc.

The detection principle of this kit: under acidic conditions, selenium is reduced to tetravalent selenium, and then reacts with the chromogenic agent to produce yellow complex, which increase the absorbance at 420 nm, and the color development intensity is proportional to the content of tetravalent selenium in the sample.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Reducing Reagent	6 mL × 1 vial	12 mL × 1 vial	-20℃, 12 months
Reagent 2	Chromogenic Agent	Powder × 2 vials	Powder × 4 vials	-20℃, 12 months, shading light
Reagent 3	Matrix Solution	7 mL × 1 vial	14 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	20 mmol/L Standard	1 mL × 1 vial	1 mL × 2 vials	-20℃, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (415-425 nm, optimum wavelength: 420 nm), Incubator (37℃)

Reagent:

PBS (0.01 M, pH 7.4)

Consumptive material:

10kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of chromogenic working solution：

Dissolve one vial of chromogenic agent with 3 mL of matrix solution, mix well to dissolve. Store at -20°C for 3 days protected from light.

③ The preparation of reaction working solution：

For each well, prepare 200 μL of reaction working solution (mix well 100 μL of reducing reagent and 100 μL of chromogenic working solution). The reaction working solution should be prepared on spot.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 20 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 4, 8, 12, 16 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.5**	**1**	**2**	**4**	**8**	**12**	**16**
20 mmol/L standard (μL)	0	5	10	20	40	80	120	160
Double distilled water (μL)	200	195	190	180	160	120	80	40

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 100 mg tissue in 900 μL double distilled water with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection. Detect the prepared sample within 1 day.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL double distilled water with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 5 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection. Detect the prepared sample within 1 day.

Operating steps

① Standard well: add 60 μL of different concentrations of standard solutions into the wells.
Sample well: add 60 μL of filtered sample supernatant into the wells.

② Add 200 μL of reaction working solution into each well.

③ Incubate at 37°C for 30 min, and measure the OD value at 420 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue sample:

$$\text{selenium content (mmol/kg wet weight)} = \frac{\Delta A - b}{a} \times V \div m \times f$$

Cell sample:

$$\text{selenium content (mmol/10^6)} = \frac{\Delta A - b}{a} \times V \div n \times f$$

[Note]

ΔA: OD_{sample} - OD_{blank} (The OD value when the standard concentration is 0).

m: The wet weight of tissue, kg.

n: The number of cell samples, 10^6.

V: The volume of double distilled water in the preparation step of sample, L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three standard were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.00	5.00	10.00
%CV	0.3	0.4	0.32

Inter-assay Precision

Three standard were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.00	5.00	10.00
%CV	0.5	0.7	0.68

Recovery

Take three standard of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	5	10	15
Observed Conc. (mmol/L)	5.1	10.6	15.5
Recovery rate (%)	102	106	103

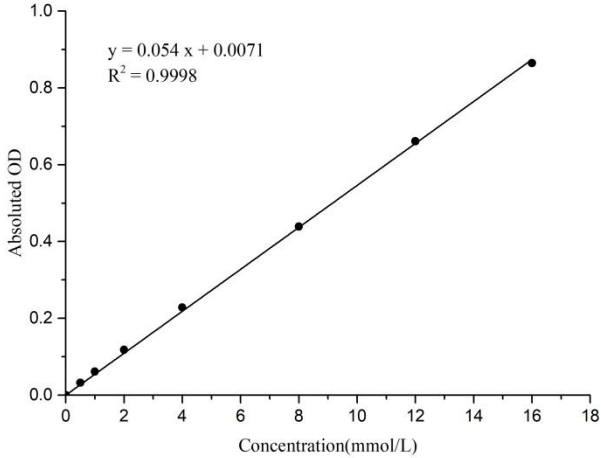
Sensitivity

The analytical sensitivity of the assay is 0.06 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0	0.5	1	2	4	8	12	16
OD	0.092	0.125	0.153	0.211	0.321	0.529	0.750	0.949
	0.093	0.125	0.155	0.210	0.321	0.534	0.757	0.965
Average OD	0.093	0.125	0.154	0.211	0.321	0.532	0.754	0.957
Absoluted OD	0	0.033	0.062	0.118	0.229	0.439	0.661	0.865



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.