This short manual is an excerpt of the manual:

For professional use only

selenOtest ELISA

For the in vitro determination of selenoprotein P in serum

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IMPORTANT NOTE

The following instructions serve as a short manual for the preparation and execution of the test. **It is important to note that this document does not replace the full instructions for use.**

1. INTENDED PURPOSE

The selenOtest ELISA is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of selenoprotein P in human serum. The test is an *in vitro* diagnostic device and is intended for use by professional users in a laboratory environment. It can be performed manually or using an automated platform. It is used to monitor functional selenium status in individuals with suspected selenium deficiency or excess and can also be used to support the differentiated assessment of selenium status in the context of selenium deficiency therapy through selenium supplementation.

2. INTRODUCTION

Selenoprotein P (SELENOP) is a multifunctional glycoprotein that plays a central role in selenium metabolism in the human body [1, 2]. It belongs to the family of selenoproteins, which contain selenium as an essential component in the form of the amino acid selenocysteine [3].

The main function of SELENOP is to ensure the transport and distribution of selenium from the liver to peripheral tissues, particularly the brain and endocrine glands, which have a high selenium requirement. SELENOP also has antioxidant and protective properties. Reduced growth, infertility of males and pronounced neurodegeneration with epileptic seizures have been observed in animal models of SELENOP deficiency [4].

Selenium is mainly absorbed through food, with selenium-rich foods such as fish, meat, eggs and milk being the primary sources. After absorption in the intestine, selenium is mainly incorporated in the liver in the form of selenocysteine in the synthesis of selenoproteins such as SELENOP. Selenium availability in the hepatocytes is the most important limiting factor [5].

An unbalanced SELENOP status has a negative effect on the immune system, the thyroid axis and cognitive and musculoskeletal performance. In addition, SELENOP deficiency increases the risk of cardiovascular events (stroke, heart attack) and tumour diseases (especially liver, intestine and kidney) [6]. An elevated SELENOP status serves as an indicator of selenium intoxication (selenosis), which can manifest itself through symptoms such as nausea, hair loss, brittle fingernails and toenails and neurological symptoms such as tingling or concentration disorders [7].

Both deficiency and excess impair the antioxidant defence, the hormone axes, the immune system and the function of vital organs. Regular monitoring of selenium status is therefore a useful diagnostic tool for monitoring and adjusting personalised care, protecting against degenerative processes and thus maintaining health [8].

3. MATERIALS PROVIDED

Cat. No.	Identifier	Kit components	Quantity	
			YC5900	YC5900.20
YC5900	PLATE	Microtiter plate, pre-coated	12 x 8 wells	20 x 12 x 8 wells
	AB	Detection antibody concentrate	1 x 300 µl	10 x 300 µl
	STD	Standard concentrate, lyophilized (see product specification for concentration)	4 x vial	20 x vial
	CTRL1	Control 1, lyophilized (see product specification for range)	4 x vial	20 x vial
	CTRL2	Control 2, lyophilized (see product specification for range)	4 x vial	20 x vial
	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 40 ml	6 x 100 ml
	READBUF	Antibody dilution buffer	4 x 6 ml	3 x 90 ml
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml	20 x 100 ml
K 0005.15	RECSOL	Reconstitution solution, ready-to-use	1 x 15 ml	5 x 15 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml	20 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml	20 x 15 ml

4. MATERIALS REQUIRED BUT NOT SUPPLIED

- Ultrapure water (conductivity ≤ 0.055 µS/cm at 25 °C, particle size ≤ 0.2 µm)
- 1.5 ml standard single-use laboratory plastic vials (disposables)
- Calibrated precision pipettes and single-use tips with variable volumes from 10–1000 µl
- Microtiter plate photometer with 450 nm filter, optionally with reference wavelength (620 nm)
- Calibrated multi-channel pipettes or multipipette and single-use tips with variable volumes from 10–1 000 µl
- Vortex mixer without specific requirements

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.**
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** must be diluted with ultrapure water **1:10** before use (e. g. 100 ml WASHBUF + 900 ml ultrapure water), mix well. Due to the high salt content in the concentrate, crystals may form. The crystals dissolve at room temperature (15–30 °C) or in a water bath at 37 °C. **WASHBUF stored at 2–8 °C** can be used until the indicated expiry date. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed bottle at **2–8 °C for 1 month**.
- The **lyophilized standard concentrate (STD)** and the **lyophilized controls (CTRL)** can be stored at **2–8 °C** and used until the specified expiration date. Reconstitute **STD** and **CTRL** with **x µl RECSOL** (x = see specification), leave to stand at room temperature (15–30 °C) for at least 5 minutes, and mix thoroughly using a vortex mixer. The selenoprotein P content varies slightly from batch to batch; the exact content is specified in the product specification. The controls are ready for use after reconstitution and do not require further dilution.
- **Standards and controls** (**STD** and **CTRL**) are stable when opened, reconstituted, or as a working solution for at least one working day **(up to 12 hours)** at **≤ 28 °C**.
- **Preparation of the detection antibody:** Add **50 µl** of **detection antibody concentrate** (**AB**) to the reagent bottle with **6 ml antibody dilution buffer** (**REABUF**), then close the bottle and mix by gently by swirling 4 to 5 times. This solution is stable for at least one working day **(up to 12 hours)** at **≤ 28 °C**.

- Remove as many microtiter strips from the kit as needed. After opening the vacuum-sealed aluminum packaging, store all unused strips together with the desiccant bag in the sealed aluminum packaging at **2–8 °C.** The strips can be used until the expiration date indicated on the label.
- Substrate solution must be colorless before use.
- Avoid foaming when mixing reagents.
- All other test reagents are ready to use and can be used until the indicated expiration date when stored at **2–8 °C** (see label).
- The reagents must not be used after the expiration date indicated on the kit packaging.
- Do not mix reagents of the test kit with other lots. Furthermore, wells of different microtiter plates, even of the same lot, must not be combined and used for analysis.
- Do not interchange plugs and caps from different reagents.
- Quality controls must be measured with each run.

Preparation of the standard curve

- A standard curve is prepared in 6 reaction vials (capacity 1.5 ml) with the **standard concentrate (STD)** and **sample dilution buffer (SAMPLEBUF)** according to the following scheme:

SELENOP [µg/l]	SAMPLEBUF [µl]	+	Standard concentrate [µl]	=	Total volume [µl]
Standard 1: 0	500	+	0	=	500
Standard 2: 15	490	+	10	=	500
Standard 3: 30	480	+	20	=	500
Standard 4: 60	460	+	40	=	500
Standard 5: 120	420	+	80	=	500
Standard 6: 240	340	+	160	=	500

6. STORAGE AND PREPARATION OF SAMPLES

6.1 Sample collection

Serum must be collected and processed using the appropriate blood collection tubes in accordance with the manufacturer's instructions.

Blood collection tubes containing liquid anticoagulants have a diluting effect, resulting in lower values for patient samples. To minimise dilution effects, it is important that the appropriate blood collection tubes are completely filled according to the manufacturer's instructions.

6.2 Sample storage

Samples are stable for up to 1 day at ≤ 25 °C, 8 days at 2–8 °C, or 6 months at –20 °C. Up to three freeze–thaw cycles have no effect on the measured selenoprotein P concentration.

6.3 Sample dilution

Serum samples are diluted 1:41 with sample dilution buffer (**SAMPLEBUF**):

e. g. **10 µl** sample + **400 µl** SAMPLEBUF

100 µl of the dilution are used per well in the test.

7. ASSAY PROCEDURE

7.1 Test principle

This assay is a sandwich ELISA for the quantitative measurement of selenoprotein P in human serum.

The wells of the microtiter plate are coated with a monoclonal anti-selenoprotein P-antibody. During the first incubation step, selenoprotein P present in the sample is bound to the immobilized antibody, forming the first antigen-antibody complex. After a washing step to remove all unbound sample components, a horseradish peroxidase (HRP)-labeled detection antibody is added. A capture antibody-antigen-detection antibody-complex is formed. Unbound substances are removed by another washing step. After the addition of an enzymatic reaction substrate (tetramethylbenzidine, TMB), HRP catalyzes the oxidation of the colorless TMB into blue oxidized TMB. The reaction is stopped by adding an acidic stop solution and the color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of SELENOP Results are determined via a calibration curve.

7.2 Automated processing

For automated processing of the test, automation-specific adjustments must be made to the test procedure in order to fulfil technical requirements. For support and queries, please contact your provider or Immundiagnostik AG.

7.3 Manual processing

Before use, allow all reagents and samples to reach room temperature (15–30 °C) and mix well.

Mark the positions for standards/samples/controls in the protocol sheet. Remove the required microtiter strips from the kit.

Take as many microtiter strips as needed from the kit.

Pipetting standards/samples/controls is to be carried out **in duplicate**.

1.	Pipette **100 µl standards/controls/diluted samples** into the respective wells.
2.	Incubate for **45 min** at room temperature **(15–30 °C)**.
3.	Discard the content of each well and wash **5 x with 250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
4.	Add **100 µl detection antibody** (with **REABUF** diluted **AB**) into each well.
5.	Incubate for **30 min** at room temperature **(15–30 °C)**.
6.	Discard the contents of the wells and wash each well **5 x with 250 µl wash buffer**. After the final washing step, remove any remaining wash buffer by tapping the plate onto absorbent paper.
7.	Add **100 µl substrate** (**SUB**) into each well.
8.	Incubate for **25–30 min*** at room temperature **(15–30 °C)** in the **dark**.
9.	Add **100 µl** stop solution (**STOP**) into each well.
10.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm as a reference. If no reference wavelength is available, read only at 450 nm.

* The intensity of the color change is temperature sensitive. We recommend observing the color change and stopping the reaction upon good differentiation

8. WARNINGS AND PRECAUTIONS

- The assay should only be performed according to the instructions provided with the kit.
- As a precaution, Iit is recommended that the kit components always should be handled as potentially infectious material.
- The kit components contain ProClin™ 950 or ProClin™ 300 to protect against bacterial contamination. ProClin™ 950 and ProClin™ 300 may cause allergic reactions in contact with skin. **Warning:** May cause an allergic skin reaction (H317). Wear protective gloves, protective clothing, eye protection/face protection while using the kit components. **IF ON SKIN:** Wash with plenty of water. If skin irritation or rash occurs: Get medical advice/attention. Substrates for enzymatic color reactions are also described as toxic and carcinogenic. Any contact with skin or mucous membranes should be avoided.
- The antibody concentrate (**AB**) contains CMIT/MIT (3:1) and MIT to protect against bacterial contamination. CMIT/MIT (3:1) and MIT are harmful to aquatic life with long-lasting effects (H412). Avoid release into the environment.
- The 10 x Wash Buffer Concentrate **(WASHBUF)** contains detergents which may cause severe irritation upon contact with the eyes.
  Warning: Causes severe eye irritation (H319). **IN CASE OF CONTACT WITH EYES**: Rinse cautiously with water for several minutes. If possible, remove contact lenses. Continue rinsing. If eye irritation persists: Seek medical advice/attention.
- The stop solution consists of diluted sulphuric acid (H_2SO_4). H_2SO_4 is a strong acid and must be used with care even in diluted form. H_2SO_4 causes burns on contact with the skin. It should therefore be handled with protective gloves, protective clothing and safety goggles. In case of contact with the acid, the burnt area must be rinsed immediately with plenty of water. Do not inhale vapors and avoid inhalation. May be corrosive to metals (H290). Keep only in original container.
- In the event of warranty claims, the rejected material must be returned to the manufacturer, Immundiagnostik AG, accompanied by a written explanatory statement within 14 days.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- This kit has been manufactured and placed on the market in accordance with Regulation (EU) 2017/746 (IVDR).

9. LITERATURE

1. Burk, R.F. and K.E. Hill, Selenoprotein P-expression, functions, and roles in mammals. Biochim Biophys Acta, 2009. 1790(11): p. 1441-7.
2. Schomburg, L., Selenoprotein P - Selenium transport protein, enzyme and biomarker of selenium status. Free Radic Biol Med, 2022. 191: p. 150-163.
3. Atkins, J.F. and R.F. Gesteland, The twenty-first amino acid. Nature, 2000. 407(6803): p. 463, 465.
4. Schweizer, U., et al., Seizures, ataxia and parvalbumin-expressing interneurons respond to selenium supply in Selenop-deficient mice. Redox Biol, 2022. 57: p. 102490.
5. Ha, H.Y., et al., From Selenium Absorption to Selenoprotein Degradation. Biol Trace Elem Res, 2019. 192(1): p. 26-37.
6. Schöttker, B., et al., Strong associations of serum selenoprotein P with all-cause mortality and mortality due to cancer, cardiovascular, respiratory and gastrointestinal diseases in older German adults. Eur J Epidemiol, 2024. 39(2): p. 121-136.
7. Brodin, O., et al., Selenoprotein P as Biomarker of Selenium Status in Clinical Trials with Therapeutic Dosages of Selenite. Nutrients, 2020. 12(4).
8. Rayman, M.P., Selenium and human health. Lancet, 2012. 379(9822): p. 1256-68.

10. SYMBOL EXPLANATION

LOT	Lot number	REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device	→REF	To be used with
	Manufacturer		Contains sufficient for <n> tests
	Temperature limitation		Use by
	Consult product specification data sheet		Consult instructions for use
	European Conformity	UDI	Unique Device Identification
	Contains plasma derivates or human blood		Warning: For associated hazard statements please see chapter PRECAUTIONS
	Corrosive		