DE Die deutsche Version der ARBEITSANLEITUNG ist als Download verfügbar unter: http://www.ici-diagnostics.com/products/selenotest-elisa/

PRODUCT INFORMATION AND INSTRUCTIONS FOR USE

(Revision 20170303_EN
Replaces: Revision 20150626_EN)

Selenotest ELISA

Version 4

Colorimetric enzyme immunoassay for the quantitative measurement of human selenoprotein P in serum

CE

REF STE

Σ 96

+2°C +8°C

6 month after delivery

INTENDED USE

The Selenotest ELISA was developed and validated for the quantitative measurement of human selenoprotein P (SELENOP, SeP, SELP, SEPP1, P49908) concentrations in the diluted serum samples.

PRODUCT DESCRIPTION

The Selenotest ELISA [1,2] is a chromogenic enzyme-linked immunosorbent assay for the quantitative determination of human selenoprotein P [3] in serum samples.

The Selenotest ELISA is a sandwich enzyme immunoassay in 96 well plate format and uses two different selenoprotein P specific monoclonal antibodies for the antigen capture and detection steps.

The Selenotest ELISA kit include an antibody pre-coated and pre-blocked microplate in a ready-to-use state, pre-diluted calibration and control samples and additional components required for assay developing.
The Selenotest ELISA are uses 8 calibrator serum samples (6 calibration and 2 anchor point) and 3 control serum samples (low, medium, high). The calibrator and control samples can be placed at any position into the assay plate.
The selenoprotein P concentrations of the calibrators and controls will be determined by measurements against serial dilutions of NIST SRM 1950 Standard Reference Material [4,5,6] for the respective Selenotest ELISA kit batch. The quantification range of selenoprotein P is a function of the respective calibration and approximately between 400 ng and 10 ng per mL. This corresponds to a selenoprotein P concentration range of 10.0 micrograms to 0.5 micrograms per mL in undiluted serum samples.
72 serum samples in single determinations or 36 serum samples in duplicate determinations can be measured within a Selenotest ELISA assay plate. The samples need to be diluted in sample dilution buffer for the measurement. The recommended sample dilution factor will be determined from the EC_{50} value of the current calibration. Per single determination between 5 and 10 microliters of serum sample will be required.

The assay is carried out according to a standard ELISA protocol at ambient temperature on a microplate shaker. The assay can be performed manually or semi-automated.
The Selenotest ELISA uses a TMB-based chromogenic substrate, which is measured at a wavelength of 450 nm after stopping the detection reaction.
The absorbance of the developed dye is directly related to the amount of human selenoprotein P contained in the analysed serum sample.

The test requires an execution time of approximately 5 to 6 hours. At least 6 assay plates can be processed by a laboratory technician in parallel on a working day.
The Selenotest ELISA assay protocol was developed for the analysis of large sample sizes e.g. from clinical biomarker studies.

The Selenotest ELISA was developed in compliance with FDA-, ICH- and expert group recommendations [7,8,9,10] to meet the quality demands of clinical biomarker studies and was validated in a multi-laboratory study using blood serum from Caucasian donors.
In addition, more than 20,000 healthy and pathological human serum samples were analysed using the Selenotest ELISA to confirm the validity and reliability of the test.

A Selenotest ELISA reference list is available for download from: http://www.ici-diagnostics.com/products/selenotest-elisa/

CE CONFORMITY MARKING

The Selenotest ELISA kit fulfils the obligations imposed by annex III sections 2 to 5 of the directive 98/79/EC. The CE conformity marking was applied in accordance to article 16 and annex X of the directive 98/79/EC.

The manufacturer EC declaration of conformity is available for download in pdf-file-format:
http://www.ici-diagnostics.com/products/selenotest-elisa/

The Selenotest ELISA is categorized according to the MEDDEV 2.14/2 rev.1 IVD guidance: Research Use Only Products, section 05, paragraph C, as a product intended for the identification and quantification of individual chemical substances or ligands in biological specimens for research purposes.
Within this scope the Selenotest ELISA can be used by research, pharmaceutical and medical-diagnostics laboratories.

InVivo BioTech Services GmbH
Neuendorfstr. 24a
16761 Hennigsdorf
Germany
phone: +49 3302 883 769
fax: +49 3302 883 771
email: info@invivo.de
web: www.invivo.de

ISO 9001:2008 Certified Company
Registration No. 320103 QM08

ASSAY PRINCIPLE

The Selenotest ELISA is based on the sandwich ELISA principle. Monoclonal antibodies against human selenoprotein P are bound to the surface of an assay plate.
To be examined serum samples are pre-diluted into a sample dilution buffer and transferred along with the reconstituted calibrator and control samples to the assay plate. Selenoprotein P from the sample is bound by the antibody to the assay plate.
In the next step, a second biotin-labelled human selenoprotein P monoclonal antibody is added, that binds to the selenoprotein P captured by the first antibody in the assay plate.
In the subsequent step, streptavidin conjugated to horseradish peroxidase (HRP) is added, which binds to the biotin-label of second selenoprotein P antibody.
In the final step, a TMB-based peroxidase substrate is added and a blue-appearing reaction product is formed. The enzymatic reaction is stopped by adding diluted sulphuric acid. The change of the pH value induces a shift in the absorption spectrum of the formed reaction product. The light absorbance of the now yellow-appearing dye is measured by a microplate reader at a wavelength of 450 nm.
The light absorbance of the developed dye is directly related to the amount of human selenoprotein P contained in the sample.

PERFORMANCE CHARACTERISTICS

Every released Selenotest ELISA batch is quality tested.
During quality testing the main performance characteristics based on specified requirements of FDA-, ICH- and expert group recommendations [7,8,9,10] is determined.

A quality certificate containing the batch specific results of the quality testing and assay performance evaluation is enclosed in each test kit package and additionally available for download:
http://www.ici-diagnostics.com/products/selenotest-elisa

Assay validation results and the analytical performance characteristics of the Selenotest ELISA were published in:
HYBSIER, S., T. SCHULZ, Z. WU, I. DEMUTH, W.B. MINICH, K. RENKO, E. RIJNTJES, J. KÖHRLE, C.J. STRASBURGER, E. STEINHAGEN-THIESSEN and L. SCHOMBURG. Sex-specific and inter-individual differences in biomarkers of selenium status identified by a calibrated ELISA for selenoprotein P. *Redox Biology*. 2017, **11**, 403-414. http://dx.doi.org/10.1016/j.redox.2016.12.025

LIMITATIONS

1 The performance of this device has not been evaluated for immunocompromised individuals.

2 This device has been evaluated for the measurement of human selenoprotein P only.

3 This device has been evaluated for use with human serum only.

4 The calibration is valid for measurement of human selenoprotein P human in serum samples only.

5 Autoantibodies against human selenoprotein P can lead to erroneous results.

6 Heterologous antibodies against mouse immunoglobulins can lead to erroneous results.

REFERENCE RANGES

The Selenotest ELISA is offered exclusively for the use for research purposes.
While the diagnostic application of selenoprotein P is still subject of clinical research, the assay cannot be validated for *in-vitro* diagnostic use.
Therefore, normal and pathological reference ranges has to be determined by the user itself if it is essential for specific clinical statements.

CONTENT AND HANDLING NOTES

1	SORB AB	**Assay plate** **1 pc.** selenoprotein P antibody pre-coated and pre-blocked microplate, ready to use Note: The assay plate should be removed from the foil pouch immediately before use. The foil pouch should not be opened before room temperature has been reached, so that no condensation of moisture in the wells of the assay plate occurs.
2	CAL 1 CAL 2 CAL 3 CAL 4 CAL 5 CAL 6 CAL 7 CAL 8	**Calibrator 1, 2, 3, 4, 5, 6, 7, 8** **0.5 mL** calibrator serum samples, lyophilised Note: Contain human serum and ProClin 950. Please consider to safety notes 2 and 3.
3	CONTROL L CONTROL M CONTROL H	**Control L, M, H** **0.5 mL** control serum samples, lyophilised Note: Contain human serum and ProClin 950. Please consider to safety notes 2 and 3.
4	CONTROL B	**Control B** **0.5 mL** assay blank, lyophilised Note: Contains ProClin 950. Please consider to safety note 3.
5	CONJ AB	**Detection Antibody** **0.15 mL** concentrate of the biotin-labelled detection antibody
6	CONJ EN	**Enzyme Conjugate** **0.15 mL** concentrate of the streptavidin-peroxidase conjugate
7	BUF RCNS	**Reconstitution Buffer** **8 mL** ready-to-use solution for reconstitution of the lyophilized calibrators and control samples Note: Contains ProClin950. Please consider to safety note 3.
8	DIL AB	**Antibody Dilution Buffer** **12 mL** ready-to-use solution for diluting of detection antibody concentrate Note: Contains ProClin950. Please consider to safety note 3.
9	DIL EN	**Conjugate Dilution Buffer** **12 mL** ready-to-use solution for diluting of enzyme conjugate concentrate Note: Contains ProClin950. Please consider to safety note 3.
10	DIL SPE	**Sample Dilution Buffer** **22 mL** buffer for pre-dilution of serum samples, ready-to-use Note: Contains ProClin950. Please consider to safety note 3.
11	BUF WASH 10×	**10× Wash Buffer** **50 mL** concentrate for preparation of 500 mL washing buffer Note: Contains ProClin950. Please consider to safety note 3. Occasionally the concentrated wash buffer tends to precipitate during storage. The precipitates dissolve at room temperature or after diluting to the working solution and do not influence the buffer performance.
12	SUBS TMB	**TMB Substrate** **12 mL** Peroxidase substrate solution (TMB), ready-to-use Note: The substrate solution is light sensitive and should be decanted into a reagent reservoir immediately before use. The substrate solution is colourless before the reaction and the reaction product is of blue colour. After stopping the reaction with sulphuric acid the absorbance spectrum of the dye is altered by the changing of the pH value. The dye will now appear yellowish.
13	SOLN STOP	**Stop Solution** **12 mL** diluted sulphuric acid, ready-to-use  **Warning** Sulphuric acid is a strong acid and must be handled with care in diluted state too. Therefore it should be worked with safety gloves, protective clothing and goggles. After contact with acid flush with plenty of water immediately. The MSDS is available for download from: http://www.ici-diagnostics.com/products/selenotest-elisa/ Hazard statements: H315 Causes skin irritation. H319 Causes serious eye irritation. Precautionary statements: P264 P280 P302+P352 P305+P351+P338 P332+P313 P337+P313 P362
14 -		**Plate Cover Foils** **2 pcs.** for airtight sealing of assay plate during incubation steps Note: The cover foils can be reused. If the foil was contaminated then the foil should be replaced to avoid carry-over to the next incubation step.
15 -		**Instructions for use** **1 pc.**
16 -		**Specification data sheet** **1 pc.**
17 -		**Quality certificate** **1 pc.**

WARNINGS AND PRECAUTIONS

1 The test kit is intended for professional use by trained laboratory personnel only.
Care should be taken to comply with the recommendations for good laboratory practice and the current local laboratory safety guidelines. If required laboratory protective clothing, disposable gloves and protective goggles should be worn during the test execution.

2 The human serum used for the calibrators and control samples has been tested negative for HIV and HCV antibodies. The serum was non-reactive for HBsAg, HIV-1 RNA, HCV RNA. Negative in a serological test for syphilis.
Nevertheless, it is recommended to handle these components with precaution as potentially infectious materials and to work in compliance with the occupational health and safety measures and disposal regulations for infectious material.

3 Some components contain ProClin950 (2-methyl-4-isothiazolin-3-one) as protective agent against microbial contamination. ProClin950 may have a sensitizing effect. The contact of test kit reagents with the skin or mucous membranes should be avoided.

ADDITIONAL MATERIAL REQUIRED, BUT NOT SUPPLIED WITH THE KIT

1 10, 100, 1000 µL pipettes with adjustable volume

2 100, 300 µL 8- or 12 channel pipettes (variable volume) or multi-channel dispenser pipette

3 Reagent reservoirs for multichannel pipettes

4 Polypropylen 96-well microplate or tubes for diluting samples

5 Vortex mixer for microtubes

6 Centrifuge for 1.5 and 2 mL microtubes

7 Microplate washer (option)

8 Microplate shaker

9 Microplate reader capable measuring absorbance at 450 nm

10 Absorbent paper towels

11 Aluminium foil for light protection of assay plate during TMB substrate incubation step (option)

12 Laboratory water (type 1 or 2)

13 Data analysis and graphing software

STORAGE AND STABILITY

All test kit components should be stored unopened until use in a refrigerator at temperatures between +2°C to +8°C and are stable at these storage conditions up to the specified expiration date printed on the test kit package, on the quality certificate and on the product specification sheet.
Prior usage the test kit should be removed from the cold storage and all components equilibrated to laboratory ambient temperature. All kit components are stable during a working day of 8 - 12 hours at room temperature (20°C to 28°C).
The working solution of the enzyme conjugate must not be frozen!

TABLE Storage stability of the unopened Selenotest ELISA kit, reconstituted calibrators, control samples and the prepared working solutions

Temperature	<-16°C	+5°C	+25°C	+37°C
Selenotest ELISA kit	up to 48 month	up to 6 month	up to 28 days	up to 5 days
Calibrators and Control Samples, reconstituted	up to 12 month	up to 72 h	up to 24 h	up to 2 h
Detection Antibody, diluted	not recommended	up to 72 h	up to 12 h	not recommended
Enzyme-Conjugate, diluted	not recommended	up to 72 h	up to 12 h	not recommended
Washing Buffer	indefinite	up to 28 days	up to 7 days	up to 24 h

SAMPLE MATERIAL UND PREPARATION

The Selenotest ELISA has been validated for the use of human serum as sample matrix for the measurement of selenoprotein P.
Serum specimens should be processed and frozen immediately after collection. Serum samples for the measurement of selenoprotein P can be stored frozen indefinitely. For sample storing periods of longer than 1 year the storage at temperatures lower than -60°C is recommended.
Repeated freezing and thawing of serum samples is possible and has no significant influence on the measured selenoprotein P concentration of the sample.
Serum samples should always be stored tightly sealed to prevent loss of volume through evaporation resulting in higher concentration recovery of the analyte.
Thaw the serum samples before selenoprotein P measurements completely at room temperature and mix well.
Serum samples for the determination of selenoprotein P using the Selenotest ELISA may not be stored in the refrigerator or at temperatures higher then room temperature.
Selenoprotein P for the measurement in Selenotest ELISA is stable up to 24 hours at room temperature (20°C to 28°C) in human serum.
Centrifuge briefly before opening the sample containers.
The samples need to be pre-diluted in sample dilution buffer. See ASSAY PROCEDURE steps 4 and 5.
The sample dilution factor is specific for the respective test kit batch and is stated on the PRODUCT SPECIFICATION SHEET along with the recommended pipetting volumes.
From each serum sample 2 separate dilutions (duplicate) should be prepared into a sample dilution microplate, transferred to the assay plate and analyzed. In this way, dilution and handling failures of various causes can be identified.

TABLE Storage stability of serum samples and diluted serum samples for the immunological analysis using the Selenotest ELISA

Temperature	<-60°C	<-16°C	+5°C	+25°C	+37°C
Serum, undiluted	indefinite	up to 12 month	not recommended	up to 24 h	up to 15 min
Serum, diluted into sample buffer	indefinite	up to 12 month	up to 72 h	up to 24 h	up to 2 h

PREPARATION OF REAGENTS

All components of the test kit should be equilibrated to room temperature before use. All reagents can be removed along with the foam insert from the packaging. Thus, no additional tube-stand is required, and the processing of the assay steps is facilitated.
The wash buffer is prepared by dilution of the wash buffer concentrate. The lyophilized calibrators and control samples are reconstituted and the detection antibody concentrate and enzyme conjugate concentrate will be each diluted into theirs appropriate buffer solution. All other reagents are provided ready-to-use.

2	CAL 1 CAL 2 CAL 3 CAL 4 CAL 5 CAL 6 CAL 7 CAL 8	**Calibrators 1, 2, 3, 4, 5, 6, 7, 8** To each lyophilized Serum Calibrator Sample 0.5 mL of Reconstitution Buffer will be given and allowed to stand for at least 5 min at room temperature, then mixed by vortexing for 2 to 3 seconds, and centrifuged briefly. The reconstituted calibrators are stable for up to 24 h @ RT.
3	CONTROL L CONTROL M CONTROL H	**Controls L, M, H** To each lyophilized Control Serum Sample 0.5 mL of Reconstitution Buffer will be given and allowed to stand for at least 5 min at room temperature, then mixed by vortexing for 2 to 3 seconds, and centrifuged briefly. The reconstituted controls are stable for up to 24 h @ RT.
4	CONTROL B	**Control B** To each lyophilized Control B 0.5 mL of Reconstitution Buffer will be given and allowed to stand for at least 5 min at room temperature, then mixed by vortex for 2 to 3 seconds, and centrifuged briefly. The reconstituted sample is stable for up to 24 h @ RT.
5	CONJ AB	**Detection Antibody** Transfer 100 µL Detection Antibody concentrate to the bottle containing 12 mL Antibody Dilution Buffer, close the bottle and mix 4-5 times by smooth rotating (avoid foaming, don't shake strong). Use within 8 hours.
6	CONJ EN	**Enzyme Conjugate** Transfer 100 µL Enzyme Conjugate concentrate to the bottle containing 12 mL Conjugate Dilution Buffer, close the bottle and mix 4-5 times by smooth rotating (avoid foaming, don't shake strong). Use within 8 hours.
11	BUF WASH 10×	**Washing Buffer** 50 mL of 10× Wash buffer concentrate are diluted into 450 mL of laboratory water and mixed. The washing buffer can be stored at room temperature and used for up to 5 days.

ASSAY PROCEDURE

1.	**Start**	Remove the test kit from cold storage and prepare the additional required materials and tools
2.	**Sample preparation**	Thaw the serum samples (see section: SAMPLE MATERIAL AND PREPARATION), go next to step 3 while samples thawing
3.	**Reagent preparation** (please consider assay procedure note 1)	Reconstitute Calibrators and Control Samples and prepare Detection Antibody, Enzyme Conjugate and Washing Buffer working solutions (see section: PREPARATION OF REAGENTS)
4.	**Filling of the sample dilution plate with sample dilution buffer**	Fill Sample Dilution Buffer into rows C1-12, D1-12, E1-12, F1-12, G1-12 and H1-12 of the sample dilution microplate according to the recommendations given on the PRODUCT SPECIFICATION SHEET
5.	**Samples dilution** (please consider assay procedure note 2)	Dilute serum samples in duplicate into pre-filled sample dilution plate according to recommendations given on the PRODUCT SPECIFICATION SHEET
6.	**Filling of sample dilution plate with calibrators and control samples**	Add 150 to 200 µL of the reconstituted control samples and calibrators per well to rows A1-12, B1-12 of sample dilution plate according to the pipetting table below
7.	**Mixing the sample dilutions**	Shake the filled sample dilution plate and mix samples for at least 30 seconds on a microplate shaker @ 600-700 rpm
8.	**Transfer the samples to the assay plate**	Transfer 100 µL of each sample dilution, calibrators, controls from the sample dilution plate in columns or rows into the assay plate using an 8- or 12-channel pipette
9.	**Sample incubation**	Seal the assay plate with plate cover foil and incubate on a microplate shaker for 60 minutes @ 600-700 rpm and 25°C (or RT)
10.	**Washing** (Please consider assay procedure note 4)	Discard the content of each assay plate well; wash each well of the assay plate 4 times by dispensing 250 µL of washing buffer into each well and discard; remove residual buffer by tapping the plate on absorbent paper
11.	**Addition of the Detection Antibody**	Add 100 µL of detection antibody working solution to each assay plate well
12.	**Detection Antibody incubation**	Seal the assay plate with plate cover foil and incubate on a microplate shaker for 60 minutes @ 600-700 rpm and 25°C (or RT)

13. Washing (Please consider assay procedure note 4)	Discard the content of each assay plate well; wash each well of the assay plate 4 times by dispensing 250 µL of washing buffer into each well and discard; remove residual buffer by tapping the plate on absorbent paper
14. Addition of the Enzyme Conjugate	Add 100 µL of Enzyme Conjugate working solution to each assay plate well
15. Enzyme Conjugate incubation	Seal the assay plate with plate cover foil and incubate on a microplate shaker for 60 minutes @ 600-700 rpm and 25°C (or RT)
16. Washing (Please consider assay procedure note 4)	Discard the content of each assay plate well; wash each well of the assay plate 4 times by dispensing 250 µL of washing buffer into each well and discard; remove residual buffer by tapping the plate on absorbent paper
17. Addition of TMB Substrate	Add 100 µL TMB Substrate solution to each assay plate well
18. TMB Substrate incubation	Seal the assay plate with plate cover foil and incubate on a microplate shaker for 60 minutes @ 600-700 rpm and 25°C (or RT) protected from light (if necessary, cover with aluminium foil)
19. Reaction stop (Please consider section HANDLING NOTES.)	Add 100 µL Stop Solution to each well and mix shortly onto a microplate shaker
20. Read absorbance at 450 nm	Read the absorbance of the developed yellowish dye at a wavelength of 450 nm by use of a microplate reader within 15 min after addition of the Stop Solution

Example – Sample dilution and assay plate pipetting table

	1	2	3	4	5	6	7	8	9	10	11	12
A	CON L	CON M	CON H	CON B	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6	CAL 7	CAL 8
B	CON L	CON M	CON H	CON B	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	CAL 6	CAL 7	CAL 8
C	S1	S2	…	…								
D	S1	S2	…	…								
E	…	…										
F	…	…										
G												
H												

Legend:
CONL, M, H = Control samples L, M, H
CON B = Control B
CAL1-8 = Calibrator 1, 2, 3, 4, 5, 6, 7, 8
S1, S2, … = Serum sample

NOTES ON ASSAY PROCEDURE

1 Reagents from different product batches cannot be mixed or interchanged, since the components of a kit batch were harmonized in order to ensure high reproducibility and optimal assay performance.
Reagents from Selenotest ELISA kits of the same batch can be mixed or interchange within the indicated self-life.

2 The sample dilutions may not be made directly into the assay plate. A polypropylene microplate or tubes should be used for pre-diluting of samples. The calibrators and controls should be pre-pipetted into the sample dilution microplate. Afterwards 100 µL of each diluted sample, calibrator and control will be transferred from the sample dilution plate to the assay plate using an 8 - or 12-channel pipette. This ensures a uniform incubation time for each sample per well. Avoid any time shift during transfer of samples or dispensing of reagents into the assay plate.

3 Create an own standard curve for each assay plate. Standard curves from other plates cannot be used for evaluation, because every assay plate has their own performance characteristics depending on the operator and conditions (as temperature, prepared working, solutions, pipetting volumes, incubation times).

4 The washing steps (see assay procedure steps 10, 13, 16) can be performed manually using an 8 - or 12-channel pipette or dispenser or automatically on an 8 - or 96-channel microplate washer.
During manual washing all wells of the assay plate are filled with wash buffer, and thereafter the wash buffer is discarded over a sink or a drip tray and the assay plate is tapped out on absorbent paper prior to re-filling.
If washing the assay plates using a microplate washer, then residual liquid is removed after the final washing step by tapping the microplate on absorbent paper.

5 One plate cover foil can be used for all incubation steps. The foil should be replaced if contaminated.

6 Avoid light exposure of the TMB substrate solution. Decant the solution directly before use into the reagent reservoir and incubate protect from light.

EVALUATION OF RESULTS

For the duplicate values of the absorbencies of the calibrators, controls and serum samples within an assay plate the mean values will be calculated. The blank value (Control B) is for control only (background signal) and must not be divided by the measured values.
The evaluation of results will be performed by a 4 - or 5-parameter logistic curve-fit function (4-PL or 5-PL) using a statistical computer software.
The selenoprotein P concentrations of the calibrators and controls are batch specific and listed on the respective PRODUCT SPECIFICATION SHEET.
The calculated selenoprotein P concentrations must be multiplied by the sample dilution factor to obtain the selenoprotein P concentration in the undiluted serum.
If the calculated selenoprotein P concentrations of the diluted samples are outside the quantitation range of the current calibration (see PRODUCT SPECIFICATION SHEET) the sample(s) must be diluted more or less. Serum samples have to be pre-diluted at least 1:8 in sample diluent for the analysis in Selenotest ELISA. Sample dilutions in the range of 1:20 to 1:60 give optimal results.

QUALITY CONTROL AND TEST VALIDITY

Based on the determined selenoprotein P concentrations of the control samples and the back-calculated selenoprotein P concentrations of the calibrated standards the accuracy of curve fitting and analysis should be verified for each assay plate following the evaluation of measuring results.
The obtained selenoprotein P concentrations must be within the valid recovery range stated on the PRODUCT SPECIFICATION SHEET.
The control samples provided with the test kit are exclusively for verifying the accuracy of the standard curve.
The internal quality assurance should be carried out using appropriate control samples that are analysed additionally to the donor serum samples. These control samples have to be similar to the composition of the analysed samples. The concentrations of the control samples should be recovered within a range of ± 30% CV.
The relative standard deviation of the duplicate values should be lower than 15% CV. Otherwise a pipetting error must be supposed and the measurement should be repeated.
The internal quality control should be designed according to the guidance "Richtlinie der Bundesärztekammer zur Qualitätssicherung labormedizinischer Untersuchungen, Teile A und B1" [11] or similar accepted guidelines for the quality control of quantitative tests in medical laboratories.

CHANGES TO THE PREVIOUS SELENOTEST ELISA KIT VERSION

1 CE marking of the test kit.

2 Changes of component identifiers and labelling.

3 The wash buffer is now supplied as a 10-fold concentrate instead of the 20-fold concentrate.

CHANGES TO THE PREVIOUS DOCUMENT VERSION

1 Corrections, updates and new contents in all chapters.

WASTE DISPOSAL INFORMATION

Disposal of primary and secondary packaging material should be done according to local regulations.
The kit paper package can be disposed or recycled as waste with the EWC code 15 01 01.
The composite foil package of the test plate can be disposed or recycled as waste with the EWC code 15 01 05.
Cleaned component packages can be disposed or recycled as plastic packaging with the EWC code 15 01 02.
The used test plate, contaminated component packages and residual components or working solutions should be disposed according to the particular internal laboratory guidelines as non-hazardous chemical waste with the EWC code 18 01 06.

SYMBOLS USED ACCORDING TO EN ISO 15223-1

	EC mark
	Batch code
	Catalogue number
	Content sufficient for <n> tests
	Manufacturer
	Temperature limits
	Use by date

OTHER USED SYMBOLS ACCORDING TO EDMA GUIDELINES

AB	Antibody
CAL	Calibrator
BUF	Buffer
CONT	Contains (volume, weight)
CONTROL	Control
CONJ	Conjugate
DIL	Diluent
EN	Enzyme
QTY	Quantity
RCNS	Reconstitute with
SOLN	Solution
SORB	Sorbent
SUBS	Substrate
SPE	Specimen

ABBREVIATIONS USED

4-PL	4-parameter logistic-log function
5-PL	5-parameter logistic-log function
CE	EC mark
CV	Coefficient of variation
EC_{50}	Half maximal effective concentration
EDMA	European Diagnostic Manufacturers Association
ELISA	Enzyme-linked immunosorbent assay
EWC	European waste catalogue
FDA	U.S. Food and Drug Administration
HBsAg	Hepatitis B virus surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ISO	International Organization for Standardization
LLOD	Lower limit of detection
LLOQ	Lower limit of quantitation

MSDS	Material safety data sheet
NIST	National Institute of Standards and Technology, U.S. Department of Commerce
pc.	Piece
pcs.	Pieces
POD	Peroxidase
RE	Relative error
RNA	Ribonucleic acid
RSD	Relative standard deviation
RT	Room temperature (ambient temperature, 20°C to 28°C)
SELENOP	Selenoprotein P (SeP, SEPP1, SELP)[12]
SRM	Standard reference material
TMB	3,3',5,5'-Tetramethylbenzidine
ULOD	Upper limit of detection
ULOQ	Upper limit of quantitation

REFERENCES

[1] HYBSIER, S., T. SCHULZ, Z. WU, I. DEMUTH, W.B. MINICH, K. RENKO, E. RIJNTJES, J. KÖHRLE, C.J. STRASBURGER, E. STEINHAGEN-THIESSEN and L. SCHOMBURG. Sex-specific and inter-individual differences in biomarkers of selenium status identified by a calibrated ELISA for selenoprotein P. *Redox Biology*. 2017, **11**, 403-414. http://dx.doi.org/10.1016/j.redox.2016.12.025

[2] HYBSIER, S., Z. WU, T. SCHULZ, C.J. STRASBURGER, J. KÖHRLE, W.B. MINICH and L. SCHOMBURG. Establishment and characterization of a new ELISA for selenoprotein P. *Perspectives in Science*. 2015, **3** (1–4), 23-24. http://dx.doi.org/10.1016/j.pisc.2014.11.013

[3] BURK R.F. and K.E. HILL. Selenoprotein P-expression, functions, and roles in mammals. *Biochim Biophys Acta*. 2009, **1790** (11), 1441-1447. http://dx.doi.org/10.1016/j.bbagen.2009.03.026

[4] PHINNEY K.W., ET AL. Development of a Standard Reference Material for metabolomics research. *Anal Chem*. 2013, **85** (24), 11732-11738. http://dx.doi.org/10.1021/ac402689t

[5] BALLIHAUT G., L.E. KILPATRICK, E.L. KILPATRICK and W.C. DAVIS. Multiple forms of selenoprotein P in a candidate human plasma standard reference material. *Metallomics*. 2012, **4** (6), 533-538. http://dx.doi.org/10.1039/C2MT20059G

[6] BALLIHAUT G., L.E. KILPATRICK and W.C. DAVIS. Detection, identification, and quantification of selenoproteins in a candidate human plasma standard reference material. *Anal Chem*. 2011, **83** (22), 8667-8674. http://dx.doi.org/10.1021/ac2021147

[7] JAPAN MINISTRY OF HEALTH, LABOUR AND WELFARE. Guideline on Bioanalytical Method (Ligand Binding Assay) Validation in Pharmaceutical Development. January 2014. http://www.nihs.go.jp/drug/BMV/BMV-LBA_draft_140124_E_rev.pdf

[8] US DEPARTMENT OF HEALTH AND HUMAN SERVICES, FDA, CDER AND CVM. Guidance for the Industry: Bioanalytical Method Validation. Draft Guidance. September 2013. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107

[9] INTERNATIONAL CONFERENCE OF HARMONIZATION (ICH) OF TECHNICAL REQUIREMENTS FOR THE REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology. Q2(R1). 2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf

[10] DESILVA B., W. SMITH, R. WEINER, M. KELLEY, J. SMOLEC, B. LEE, M. KHAN, R. TACEY, H. HILL and A. CELNIKER. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. *Pharm Res.* 2003, **20** (11), 1885-1900. http://dx.doi.org/10.1023/B:PHAM.0000003390.51761.3d

[11] Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen. *Deutsches Ärzteblatt*. 2014, **111** (38), A1583-A1618. http://www.bundesaerztekammer.de/aerzte/qualitaetssicherung/richtlinien-leitlinien-empfehlungen-zur-qualitaetssicherung/labor/

[12] GLADYSHEV V.N., et al. Selenoprotein Gene Nomenclature. *J Biol Chem*. 2016, **291**(46), 24036-24040. http://dx.doi.org/10.1074/jbc.M116.756155

TECHNICAL SUPPORT

If you need more information about the Selenotest ELISA, have questions or comments, please contact us at:

InVivo BioTech Services GmbH
email: selenotest@ici-diagnostics.com
phone: +49 30 6120 1765

This document is available for download from:
http://www.ici-diagnostics.com/products/selenotest-elisa/