AccuDiag™ Testosterone Saliva ELISA Kit

REF 7095-38

IVD See External Label 2°C-8°C Σ 96 Tests

Testosterone Saliva ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive ELISA
Detection Range	0-1000 pg/mL
Sample	100 µL Human Saliva
Specificity	See Table
Sensitivity	6.75 pg/ml
Incubation Time	90 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

For the quantitative measurement of Testosterone in human saliva by an ELISA (Enzyme-Linked Immunosorbent Assay). This kit is intended for professional use only and is for laboratory use only. For in vitro diagnostic use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

LIMITATIONS RELATED TO INTENDED PURPOSE AND USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of testosterone in human saliva. The kit is not calibrated for the determination of testosterone in other specimens of human or animal origin.
4. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.

SIGNIFICANCE AND SUMMARY

Testosterone is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. Testosterone is also produced by peripheral tissues from androstenedione, which is of little physiological significance in men. However, in women about half of the circulating testosterone is derived from this origin. The action of testosterone is both androgenic and anabolic. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyperandrogenic states in females. Most of the circulating testosterone is bound to three proteins: sex hormone binding globulin (44–78%), albumin (20–54%) and cortisol binding globulin (small amount). Only about 2–3% of the total circulating testosterone remains unbound or in the free form. Only the free portion (or the non-SHBG bound fraction) of the circulating testosterone is thought to be available to tissues where it exerts its biological actions. The salivary hormone assays are advocated for their non-invasive sample collection method. Salivary testosterone is of great clinical value as it represents a filtered fraction of plasma testosterone and is independent of salivary flow rate. Many studies have suggested that salivary testosterone correlates well with either free or non-SHBG bound testosterone.

ASSAY PRINCIPLE

The Testosterone Saliva ELISA is a competitive immunoassay. Competition occurs between testosterone present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-testosterone antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of testosterone present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the blue color to a yellow color. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of testosterone in specimen samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.

5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or saliva pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use blood contaminated saliva samples.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as >1000 pg/mL. If further dilution and retesting is required, only calibrator A may be used to dilute saliva samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

Specimen Collection & Storage

Avoid sample collection within 1 hour after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before the sample is collected. Do not use blood contaminated specimens. Approximately 0.25 mL of saliva is required per duplicate determination. Rinse mouth thoroughly with water 10 minutes before the sample is collected. Collect 1–2 mL of saliva into a clean polypropylene tube without force or inducement. Saliva samples may be stored at 2-8°C for up to 24 hours or at -20°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

Specimen Pre-Treatment & Storage

Following collection, the sample must be pretreated according to the following procedure:

1. Freeze the sample for a minimum of 2 hours.
2. Thaw the sample.
3. Vortex to mix and centrifuge the sample at 2000x g for 10 minutes.
4. Carefully remove the supernatant and transfer to a new labelled tube. The supernatant will be used in the assay procedure of the test.

Do not pre-treat the calibrators and controls; they are provided in a ready to use format.

Store pre-treated saliva samples at 2-8°C for up to 24 hours or at -20°C or lower if the analyses are to be done at a later date. Samples that have been stored should be inspected to ensure they are free from precipitates before being used in the assay. If there are precipitates present, follow steps 3-4 in section 7.2 Specimen Pretreatment & Storage. Consider all human specimens as possible biohazardous materials.

REAGENTS

Materials provided with the test kit

1. Microplate

Contents:	One anti-testosterone polyclonal antibody-coated 96- well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use

Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

2. HRP Conjugate

Contents:	One bottle containing Testosterone-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
Volume:	15 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

3. Calibrator A – F

Contents:	Six bottles of calibrator containing specified testosterone concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of testosterone. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 10, 40, 120, 360, 1000 pg/mL.
Format:	Ready to Use
Volume:	Calibrator A: 4.0 mL/bottle Calibrator B-F: 1.0 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

4. Control 1 – 2

Contents:	Two bottles of control containing different testosterone concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of testosterone. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
Volume:	1.0 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

5. TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	16 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.

6. Stopping Solution

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks.
Safety:	Refer to product SDS.  **Warning**

7. Wash Buffer Concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for three weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	(X10) **Dilute 1:10 Before Use** Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

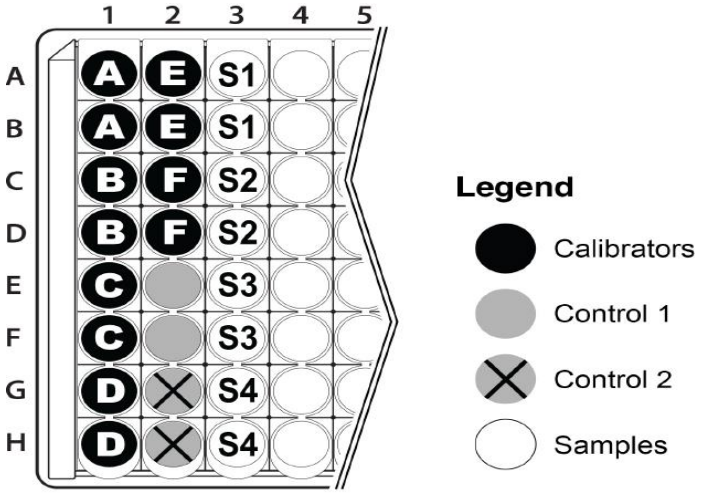
8. Microplate Film

Contents:	Microplate adhesive film.
Quantity:	2
Stability:	Stable until the expiry date printed on the label. Do not reuse.

Materials required but not provided

1. Calibrated single-channel pipette to dispense 100 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL and 150 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker:
   a. Orbital shaker (3 mm diameter) set to 600 rpm or
   b. Reciprocating shaker (1.5" stroke length) set to 180 oscillations/minute.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
9. Polypropylene tubes for sample pre-treatment (e.g., polypropylene microcentrifuge tubes).

RECOMMENDED ASSAY LAYOUT



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ASSAY PROCEDURE

Specimen Pretreatment:

All specimens that will be tested must be pre-treated before being tested (see section Specimen Pre-Treatment & Storage). Do not pre-treat the calibrators and kit controls as they are provided ready to use.

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.
2. **Prepare** the Wash Buffer Working Solution (See section Materials provided with the test kit, 7. Wash Buffer Concentrate).
3. Prepare all specimen samples that will be tested. Refer to section Specimen Pre-Treatment & Storage.
4. Plan the microplate wells to be used for calibrators, controls, and samples. See section Recommended Assay Layout. Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
5. Pipette 100 µL of each calibrator, control, and pre-treated specimen sample into assigned wells.
6. Pipette 100 µL of the HRP Conjugate into each well (the use of a multi-channel pipette is recommended). Cover the microplate with the provided Microplate Film.
7. Incubate the microplate on a microplate shaker** for 60 minutes at room temperature.
8. Remove the microplate sealing film and wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below. Automatic: Using an automatic microplate washer, perform a 3- cycle wash using 350 µL/well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid. Manually: For manual washing, perform a 3-cycle wash using 350 µL/well of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multichannel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
9. Pipette 150 µL of TMB Substrate into each well (the use of a multichannel pipette is recommended).
10. Incubate the microplate on a microplate shaker** for 30 minutes at room temperature. Do not cover the microplate.
11. Pipette 50 µL of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
12. Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

If shaking required: ** See Section Materials Required But Not Provided for microplate shaker options.

CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 1000 pg/mL and needs to be diluted and retested, then dilute with calibrator A not more than 1:4. The result obtained must be multiplied by the dilution factor.

QUALITY CONTROL

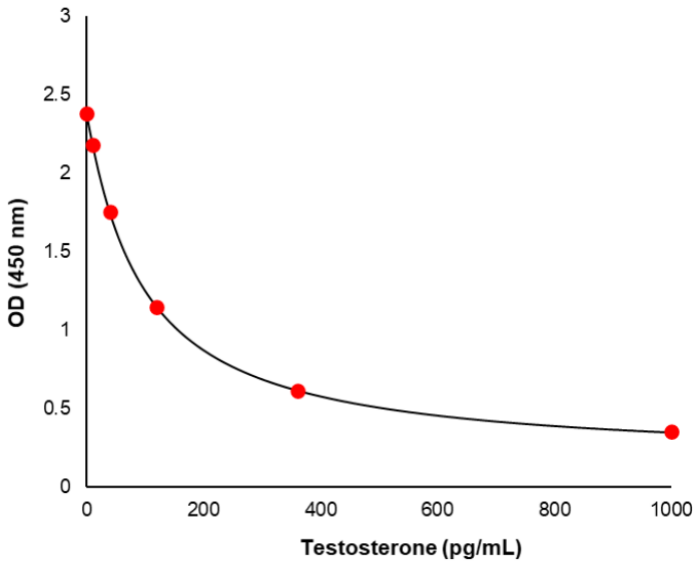
When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

TYPICAL DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.383	100	0
B	2.178	91	10
C	1.751	73	40
D	1.148	48	120
E	0.612	26	360
F	0.353	15	1000
Unknown	1.867	-	30



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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	Testosterone (pg/mL)
Limit of Blank (LoB)	2.35
Limit of Quantitation (LoQ)	6.90
Limit of Detection (LoD)	6.75

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the testosterone cross-reacting at 100%.

Compound	%Cross Reactivity
Testosterone	100
Aldosterone	0
Androstenedione	6
Androsterone	0
Cholesterol	0
Cortisone	0
Dehydroepiandrosterone	0
Dehydroepiandrosterone Sulfate	0
Dihydrotestosterone	8
Estradiol	0
Estriol	0
Pregnenolone	0
Progesterone	0

PRECISION

The precision study was performed according to the CLSI EP05-A3 guideline. The experimental protocol used a nested components-of-variance design with 10 saliva samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots per day and two replicate measurements per run (a 10 x 2 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (pg/mL)	Within Run SD (pg/mL)	Within Run CV%	Between Run SD (pg/mL)	Between Run CV%	Total SD (pg/mL)	Total CV%
1	33.5	2.3	6.8	2.7	10.7	6.0	18.0
2	64.0	3.0	4.8	6.2	9.7	8.9	14.0
3	139.2	4.3	3.1	11.7	8.4	15.9	11.4
4	142.3	5.2	3.7	14.9	10.5	17.5	12.3
5	755.9	35.4	4.7	100.0	13.2	113.2	15.0
6	631.1	32.9	5.2	70.21	11.1	79.8	12.6
7	213.3	7.7	3.6	14.2	8.4	27.9	13.1
8	40.8	2.2	5.5	5.2	12.8	6.6	16.1
9	68.9	2.1	3.0	8.0	11.7	9.8	14.2
10	167.0	6.0	3.6	14.6	8.7	20.7	12.4

LINEARITY

The linearity study was performed according to the CLSI EP06-Ed2 guideline using four human saliva samples covering the range of the assay. The samples were diluted in calibrator A up to a 1:4 dilution. Samples were tested in duplicate, and the results compared to the predicted concentrations. The statistical analysis shows that the assay is sufficiently linear up to a 1:4 dilution when using calibrator A as the diluent. The results (in pg/mL) are tabulated below.

Sample	Observed Result	Expected Result	Recovery %
1	214.4	-	-
1:2	96.0	107.2	90
1:4	46.8	53.6	87
2	341.4	-	-
1:2	139.7	170.7	82
1:4	76.7	85.4	90
3	639.2	-	-
1:2	253.6	319.6	79
1:4	173.5	159.8	109

COMPARATIVE STUDIES

The DAI Testosterone Saliva ELISA kit (y) was compared against a competitor's commercial Testosterone Saliva ELISA kit (x). The comparison of 104 human saliva samples yielded the following linear regression results:
y = -1.887 + 0.7873, r = 0.97

REFERENCE RANGES

Reference ranges (95%) were estimated using samples obtained from adult individuals. Each laboratory shall establish their own reference values.

Group	N	Median (pg/mL)	95% Confidence Range (pg/mL)
Female	40	11.3	3 - 38
Male	60	67.9	32 - 117

PRODUCT COMPLAINTS

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

WARRANTY

DAI guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

LIMITATION OF LIABILITY

DAI liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	**2022-09**
REF **7095-38**	**AccuDiag™ - Testosterone Saliva ELISA**
Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands.** www.cepartner4u.eu
Revision Date: 2022-05-13	