Total Prostate Specific Antigen (CLIA) PACKAGE INSERT

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[INTENDED USE]

Total Prostate Specific Antigen (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of total prostate specific antigen (tPSA) levels in human serum or plasma (Heparin lithium, Heparin sodium, EDTA-K_2 or EDTA-Na_2). The test is used as an aid for judging the course of the disease or the effect of treatment by dynamic monitoring of patients with prostate cancer.

The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120, and all other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

[SUMMARY]

Prostate specific antigen (PSA) is a signal-chain glycoprotein protein that has a molecular weight of 34KD. PSA has the function of serine proteinases.[1] PSA is mainly composed of prostatic acinar cells and Ductal epithelial cells. It is widely found in normal prostate tissue, benign hyperplasia and neoplastic prostate tissue, as well as prostatic fluid and semen.[2,3] There are three forms of PSA in serum. The first form of PSA is enveloped by protease inhibitor α-2 macroglobulin and has no immunoreactivity. The second form of PSA binds to protease inhibitor α-1 antichymotrypsin (ACT). The third form is free PSA (fPSA).[4,5,6] The latter two forms of PSA are referred to collectively as total PSA (tPSA). The contents of those two forms of PSA are different in different person. The concentration of fPSA may range from 5 to 50% of tPSA.[7]

Serum PSA and digital rectal examination (DRE) can be used to detect prostate cancer in men above 50 years old. Benign prostatic hyperplasia, inflammation of the prostate, or trauma may lead to PSA elevations of varying degrees. So elevated serum PSA concentration can only suggest the presence of prostate cancer until a biopsy is performed. The detection of serum PSA is also benefit for the treatment and management of prostate cancer patients. Serial measurement of PSA is useful in detecting residual tumor and recurrent cancer after radicalprostatectomy. If serum PSA concentration drops to normal level after radiation therapy, hormone therapy, or radical resection, the treatment of prostate cancer is successful.[8,9] In addition, PSA can be used as an accurate marker monitoring the advancing clinical stage in untreated patients.[10,11]

[TEST PRINCIPLE]

The Total Prostate Specific Antigen (CLIA) hereinafter also referred to as the 'tPSA' is a two-sites immunoassay sandwich method with enzyme catalytic chemiluminescence detection (CLIA).

Reagents for the assay are ready to use and pre-dispensed in the sealed reagent cartridges. All of the assay steps are performed automatically by the instrument.

The specific reaction process is as follows:

The sample was manually pipette into the sample well of reagent cartridge, then sample, sample diluent, PSA antibody labeled with alkaline phosphatase and PSA antibody coated magnetic particles are mixed thoroughly together, and the mixture is incubated. The analyte in the sample, alkaline phosphatase labeled antibody and antibody on magnetic particles were bonded together as sandwich immuno complex. The immuno complex was then washed to eliminate the unbound components in the sample. The concentration of the substance to be detected in the sample is directly proportional to the intensity of the luminescence. At the end, the results were automatically calculated by the ACCRE analyzer in relation to the calibration curve stored.

[CONTENT OF THE KIT]

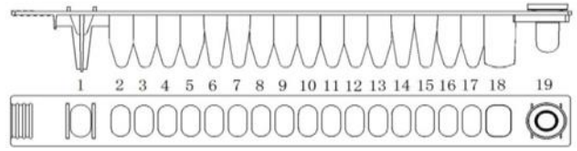
Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls. Traceability: The calibration kit has been traced to WHO International Standard Prostate Specific Antigen (human)(total: PSA-ACT + free PSA) (NIBSC code:17/100). The exact calibrator concentration values are detailed in the kit Calibrator Barcode Sheet.

Contents	Quantities		Notice
	REF W84A	REF W84C	
tPSA reagent cartridges	60 strips	36 strips	Ready to use
tPSA Calibrator 1 (CAL 1)	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, ProClin300; different concentrations of PSA antigen;
tPSA Calibrator 2 (CAL 2)	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, ProClin300; different concentrations of PSA antigen;
tPSA Quality Control Level 1 (QC 1)	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, ProClin300; different concentrations of PSA antigen;
tPSA Quality Control Level 2 (QC 2)	1.0 mL x 1 vial	1.0 mL x 1 vial	Tris buffer, ProClin300; different concentrations of PSA antigen;
Instruction for Use	1	1	N/A
Reagent Registration Card	1	1	N/A
Calibrator Barcode Sheet	1	1	N/A
Quality Control Sheet	1	1	N/A

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

The Reagent Cartridge

The reagent cartridge consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All Reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridges. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Main components of the reagent cartridge are as follows:

Well	Component	Content	Volume
7	Magnetic Particles (R1)	PSA antibody coated Magnetic Particles, Tris buffer, ProClin 300.	50μL
9	Enzyme Conjugates (R2)	ALP (alkaline phosphatase) labeled PSA antibody, Mes buffer, ProClin 300.	50μL
5	Sample Diluent (R3)	Tris buffer solution, ProClin300.	30uL
10,11 ,12	Wash solutions	Tris buffer solution, ProClin300.	350μL
16	Substrate	(4-chlorophenyl) (10-methyl-9, 10-dihydroacridine methylene) disodium phosphate salt	200μL

Note: Well 1,2,3,4,6,8,13,14,15,17,18 are empty.

[MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED]

- Sample diluent (manufactured by Tisenc), if needed.
- Pipette with disposable tip to dispense 200 μL.
- Powderless, disposable gloves.
- Disposable tips for ACCRE analyzer
- Instruments of the ACCRE series analyzers.
- For other specific materials and disposables, please refer to the Instrument User Manual.

[WARNINGS AND PRECAUTIONS]

- For in vitro diagnostic use only.
- For professional use only by qualified laboratory personnel.
- The kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest edition).
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- DO not use damaged or stained materials provided in the reagent box.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powder less gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- If you have any questions or need help, please contact the local distributor to solve problems timely.
- NOTICE TO THE USERS: Any serious incident that has occurred in relation to the Total Prostate Specific Antigen (CLIA) shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

[STORAGE]

- Store the kits upright at 2-8°C, kits can be stored for 18 months from the date of manufacture. After the reagent cartridge is taken out from the refrigerator to equilibrate, the testing should be completed within 2 hours.
- Store the calibrators and quality controls at 2-8°C till expiry date. When opened, the calibrators and quality controls will be stable for 30 days at 2-8℃.
- Keep away from sunlight.
- Do not freeze reagents cartridge.

[SAMPLES]

The sample volume for ACCRE tPSA assay is 30μL, please note that at least 55μL of sample has to be pipetted into the reagent cartridge.

Sample type and collection

Human serum or plasma (Heparin lithium, Heparin sodium, EDTA-K_2, or EDTA-Na_2).

Types of tubes:
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with heparin lithium,
- Plastic tube with heparin sodium,
- Plastic tube with EDTA-K_2,
- Plastic tube with EDTA- Na_2,

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.

For use of the sample tubes, follow the tube manufacturer's instructions for use.

The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.

Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.

Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.

If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.

Severe hemolysis samples (hemoglobin >500mg/dL), lipemia or turbidity samples are not recommended for applying on the reagents.

Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.

Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability

Samples (serum and plasma) should be tested as soon as possible. Samples can be stored at room temperature (15~30℃) for up to 8 hours or at 2~8℃ for up to 5 days; if longer storage is required, freeze the serum or plasma at -20℃±5℃ for up to 6 months, without exceeding 1 freeze/thaw cycle.

[INSTRUCTIONS FOR USE]

For complete instructions, see the Instrument User Manual.

When using the assay for the first time: Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, quality control and sample test are only available after assay was registered successfully.

When opening a new lot of reagents: Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer.

Calibration is necessary under the following conditions:

(1)New reagent lot number applied;

(2)Same lot of reagents used more than 4 weeks;

(3)Quality control out of control;

(4)When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure
1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridges. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each reagent cartridge for each sample, calibrator or control.
3. Place the reagent cartridges into the reagent rack.
4. Manually pipette the Samples, Calibrators, Controls into the sample well of reagent cartridge.
Please note that at least 55μL of sample has to be pipetted manually into the reagent cartridge and 30μL sample is needed for each test.
** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*
5. Put the sample rack into the reagent Chamber of the ACCRE analyzer.
6. Place the assay tips into the reagent Chamber of the ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. **The tPSA assay** can be performed automatically by the ACCRE analyzer.
9. After the assay is completed, remove the reagent rack from the instrument. Dispose the used reagent cartridge and assay tips into an appropriate recipient.

Dilution
Samples with concentrations above the upper limit of detection (100.000 ng/mL) can be diluted with negative serum or plasma manually or sample diluent manually. The recommended dilution is 1:50. The concentration of the diluted sample must be >2.000 ng/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control
Quality Controls is included in each Total Prostate Specific Antigen (CLIA) kit. This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. Results cannot be validated if the control value deviates from the expected values.
It is the responsibility of the user to performed Quality Control in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

[RESULTS AND INTERPRETATION]
Calculation of Results
The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 2-point calibration, and calculated according to a predefined mathematical model, the results expressed in **ng/mL**.

Interpretation of Results
The detection range is from 0.016 ng/mL to 100.000 ng/mL. For samples with tPSA below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is < 0.016 ng/mL; if the sample is higher than the upper limit of detection, the reported result is >100.000 ng/mL (if the result is higher than the upper limit of detection, then the final result is obtained by multiplying the test result after proportional dilution by the dilution factor)
Results of tPSA assays are determined by testing specimens from a study in clinical centers with group of totally 191 healthy male adult individuals in China. The test data were statistically analyzed by MedCalc. The 95th percentile reference interval is calculated.
The 95th percentile value is 4.0 ng/mL.
** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*
In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

[LIMITATIONS]
1. This test has been developed for testing human serum and plasma specimen only.
2. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
4. Please note that heterophile antibody in human serum or plasma can interfere the test result.
5. Other factors may interfere with Total Prostate Specific Antigen (CLIA) and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

[Product Performance Indexes]
Detection limit
- Limit of Blank (LoB): 0.005 ng/mL
- Limit of Detection (LoD): 0.016 ng/mL
- Limit of Quantitation (LoQ): 0.016 ng/ mL

Assessment is made in accordance with method in CLSI® EP17-A2 recommendations.
Measurement Range
The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity). **The measurement range of the Total Prostate Specific Antigen (CLIA) assay is from 0.016 ng/mL to 100.000 ng/mL.**
Reportable Range
Reportable range is defined by the analytical sensitivity and the upper limit of the master calibration curve. Assessment is made in accordance with method in CLSI® EP34 recommendations. **The reportable range of the Total Prostate Specific Antigen (CLIA) assay is from 0.016 ng/mL to 100.000 ng/mL (or the upper limit is up to 5000.000 ng/mL for 50-fold diluted samples).**
Linearity
Linearity was evaluated according to CLSI® EP06-A recommendations. The Total Prostate Specific Antigen (CLIA) assay is linear between 0.016 ng/mL to 100.00 ng/mL. The linear correlation coefficient (r) should be bigger than 0.9900.
Accuracy
Two reference samples of WHO International Standard Prostate Specific Antigen (human)(total: PSA-ACT + free PSA) (NIBSC code: 17/100) with traceable and defined value was used to verify the accuracy of this assay. The relative deviation between the detection result and the calibration concentration was within ±10%. The following results were obtained:

Sample	Detection results (ng/mL)	Calibration concentration (ng/mL)	Relative deviation
1	4.041	4.282	5.96%
2	40.173	42.265	5.21%

Precision
A precision study was performed by using Total Prostate Specific Antigen (CLIA) and four levels of clinical specimens and two levels of quality controls in a modified protocol according to CLSI® EP05-A3 recommendations: two separate runs per day for a total of 20 days in replicates of 2, using 1 lot of reagents (n=80) . The following results were obtained:

Sample	Mean value (ng/mL)	Repeatability(%CV)	Intermediate precision (%CV)	Reproducibility (%CV)
1	0.056	5.90%	2.04%	6.25%
2	4.009	6.11%	2.13%	6.47%
3	29.957	5.94%	1.51%	6.13%
4	79.699	6.05%	1.49%	6.23%
QC1	4.006	6.18%	2.27%	6.59%
QC2	59.925	6.19%	2.10%	6.54%

Interference
Potential interference substances were studied according to CLSI® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested. The following substances do not interfere with the test results at the indicated concentrations:
Endogenous Interferents

Substance	Acceptable range	Substance	Acceptable range
Hemoglobin	≤ 500mg/dL	Triglycerides	≤ 1000mg/dL
Bilirubin	≤ 10 mg/dL	Rheumatoid factor	≤ 200 IU/mL

Exogenous Interferents

Substance	Acceptable range	Substance	Acceptable range
Apatinib	≤ 0.528 mg/mL	Pabolizumab	≤ 0.429 mg/mL
Gefitinib	≤ 0.18 mg/mL	Toripalimab	≤ 0.129 mg/mL
Erlotinib	≤ 0.108 mg/mL	Tislelizumab	≤ 0.144 mg/mL
Afatinib	≤ 0.03 mg/mL	Bevalizumab	≤ 0.642 mg/mL
Aletinib	≤ 0.858 mg/mL	Cyclophosphamide	≤ 1000 mg/L
Paclitaxel	≤ 0.228 mg/mL	Fluorouracil	≤ 500 mg/L
Carboplatin	≤ 0.456 mg/mL	Methotrexate	≤ 1000 mg/L
Cisplatin	≤ 0.114 μg/mL	Tamoxifen	≤ 50 mg/L
Pemetrexed	≤ 0.57 mg/mL	Mitomycin	≤ 25 mg/L
Nedaplatin	≤ 0.114 mg/mL	Etoposide	≤ 400 mg/L
Gemcitabine	≤ 136.5 μg/mL	Flutamide	≤ 1000 mg/L
Docetaxel	≤ 0.114 mg/mL	Taxol	≤ 5.5 mg/L
Camrelizumab	≤ 0.144 mg/mL	Levothyroxine	≤ 0.24 mg/mL

Equimolar reactivity
For the monoclonal antibodies used, the following cross-reactivities were found: PSA and PSA-ACT are recognized on an equimolar basis.
HAMA
Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results[12-14].
Hook effect
High Total Prostate Specific Antigen levels can cause a paradoxical decrease in the RLUs (high dose hook effect). Total Prostate Specific Antigen levels as high as 17000ng/mL will assay greater than 100.000 ng/mL.

[WASTE DISPOSAL]
Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

[INDEX OF SYMBLOS]

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
IVD	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit	LOT	Batch code	REF	Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
	Do not roll		Do not use if package is damaged	BIO	Contains biological material of animal origin
	Biological risks		Caution	CONTROL	Control
	Upright		Fragile, handle with care		

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