RK-10CTACE200331

hPSA [I-125] IRMA KIT

(REF: RK-10CT)

The hPSA [I-125] IRMA system provides direct quantitative *in vitro* determination of human Prostate Specific Antigen (PSA) in human serum. PSA can be assayed in the range of 0-100 ng/mL using 25 µL serum samples.

Introduction

Prostate Specific Antigen (PSA) is a tissue-specific serine protease similar to the chymotrypsin- like glandular kallikreins. The active enzyme is a single chain glycoprotein of 237 amino acids (approximately 30 kDa). PSA is mainly responsible for gel dissolution in freshly ejaculated semen by proteolysis of the major gel forming proteins. The major part (70-90 %) of PSA in serum is complexed to alfa$_1$-antichymotrypsin (ACT). Total PSA (free+ACT-complex) is increased in both benign prostate hyperplasia and malignant prostate cancer.

Reliable determination of PSA has long been the subject to scientific criticism, due to special analytical difficulties encountered. Immunoassays may detect two forms of PSA in different molar ratio, a fact that may result in significiant differences of values assigned. Ideally, a reliable total PSA immunoassay is characterized by an equimolar response to both free and complex forms of PSA, contrary to those showing different immunoreactivity towards these forms ("skewed response assays"). The lack of international reference material makes it even more difficult to assign true values to PSA in serum.

The current IRMA system is characterized with an equimolar response to these two forms of PSA, and has been functionally calibrated against FDA-approved immunoassays according to recommendations of international standardization committees.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the PSA molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 1-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of PSA, the unknown concentration of PSA in patient samples can be determined.

Contents of the kit

1. 1 bottle of TRACER (11 mL), ready to use, containing about 980 kBq ^{125}I-anti-PSA and capture anti-PSA antibody in buffer with red dye and 0.1 % NaN_3.

2. 7 vials of STANDARDS (7 x 1.0 mL), ready to use, containing (S0-S6) 0; 0.1; 0.5; 2, 8; 25 and 100 ng/ml human PSA in bovine serum with 0.1% NaN_3 (Calibrated against WHO ECBS 96/670).

3. 2 vials of CONTROL SERA (2 x 1.0 mL), ready to use, human serum with 0.1% NaN_3.

The concentrations of the control sera are specified in the quality certificate enclosed.

4. 2 boxes of COATED TUBE, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (25, 100 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter.

Recommended tools and equipment

repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-ml pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens.

Storage of reagents

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date of the kit. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Use of Control Sera

Good laboratory practices require that control sera be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

Assay procedure

(For a quick guide , refer to Table 1.)

1. Label coated tubes in duplicate for each standard, control serum and samples.
2. Homogenize all reagents and samples by gentle mixing to avoid foaming.
3. Pipette **25 µL** of standards, controls and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
4. Pipette **100 µL** of tracer into each tube (Optionally, set aside 2 uncoated tubes for total counts.).
5. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm recommended).
6. Incubate tubes for **1 hour**, shaking at room temperature.
7. Add **2.0 mL** of distilled water to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
8. Return the tube-rack to an upright position, and repeat step-7 one more time.
9. Count each tube for at least 60 seconds in a gamma counter.
10. Calculate the PSA concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**25**		
Control			**25**	
Sample				**25**
Tracer	**100**	**100**	**100**	**100**
Shake for **1 hour** at room temperature				
Distilled water		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Distilled water		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}6} / C / M_x\ (cpm) - S_0\ (cpm)}{T(cpm)} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of PSA.

Determine the PSA concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	conc. (ng/mL)	Mean cpm	B/T%
T		392944	-
S0	0	292	0.00
S1	0.1	630	0.09
S2	0.5	1454	0.30
S3	2	4665	1.11
S4	8	17011	4.25
S5	25	49586	12.54
S6	100	175399	44.56
CI	3.67	8236	2.03
CII	8.73	18171	4.55

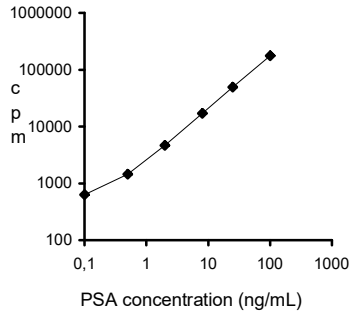

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples)*

Performance characteristics

Sensitivity

For the analytical sensitivity 0.02 ng/mL has been obtained by assaying 20 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17.

LoB = 0.035 ng/mL determined as the highest measurement result that is likely to be observed (with a stated probability [5%]) for a blank sample.
LoD = 0.09 ng/mL determined with proportions of false positives (α) less than 5 % and false negatives (ß) less than 5 %, based on 120 determinations, with 4 blanks and 6 low level samples.
LoQ = 0.12 ng/mL as graphically determined from the precision profile curve.

Precision

5 patient samples were assayed in 15 replicates to determine intra-assay precision. Values obtained are shown below.

Sample (No.)	Number of replicates	Mean value (ng/mL)	CV %
1	15	0.94	3.74
2	15	1.98	1.86
3	15	4.12	3.24
4	15	8.43	2.25
5	15	17.24	1.36

Reproducibility

To determine inter-assay precision 5 patient samples were measured in duplicates in 20 independent assays by 4 operators using different kit batches. Values obtained are shown below.

Sample (No.)	Number of runs	Mean value (ng/mL)	CV %
1	20	0.99	5.64
2	20	2.03	4.22
3	20	4.27	2.76
4	20	8.83	2.70
5	20	17.70	2.88

Specificity

Cross-reaction was measured as undetectable in the measuring ranges of hCG, hPRL, hLH, hFSH, hTSH, and AFP.

Dilution test (linearity)

For human PSA by PSA IRMA, the method has been demonstrated to be linear from 0.12 ng/mL (LoQ) to 104.1 ng/mL.

Recovery test

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking 5 serum samples with known amounts of PSA. The mean recovery obtained was 96.3% with a range: 86.13 – 103.15%.

Expected Values

Healthy adult males: < 3.0 ng/mL (n = 285).
It is recommended that each laboratory determine a reference range for its own patient population.

Age group (Years)	N	ng/mL PSA (< mean value + 3SD)
20-30	76	< 1.2
31-40	57	< 2.6
41-50	54	< 2.7
51-60	68	< 2.8
> 60	34	< 4.4

Limitations

- The reagents supplied in this kit are optimized to measure PSA levels in serum..
- The KIT has no "high-dose hook" effect with PSA levels up to 20000 ng/mL. Samples expected to have concentrations greater than the highest standard should be diluted with the zero standard and reassayed.
- The results of this assay should be used in conjunction with other pertinent clinical information.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.
2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.
3) **Addition of distilled water.** For the addition of distilled water the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information: Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1, 2), Hepatitis-C antibody (anti-HCV), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody.
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 20 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	REF	Catalogue number
IVD	In vitro diagnostic medical device	8°C 2	Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material

CE 1011

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