



**1. NAME AND INTENDED USE**

FP5A-RIACT is a kit for the radioimmunoassay of free prostate specific antigen in serum or plasma.

**2. INTRODUCTION**

PSA (Prostate Specific Antigen) is found in serum in both free and bound forms, the latter complexed to α1 anti-chymotrypsin (ACT) and to α2 macroglobulin. The PSA-ACT complex is the main form assayed in serum.

Different techniques (immunocytochemistry, western blot, serum chromatography) have enabled the variation of the ratio of free PSA to total PSA depending on the pathology observed to be brought out. This ratio is higher in cases of benign hypertrophy than in those of prostate cancer. The phenomenon may be linked to a proliferation of ACT-secreting cells in prostate cancer.

For men with a level of total PSA (PSA + PSA-ACT) between 4 - 10 ng/ml, the combination of an assay specifically measuring free PSA with one measuring total PSA (calculation of the percentage of free PSA over total PSA) is a valuable tool in the differential diagnosis of prostate cancer.

**3. PRINCIPLE**

FP5A-RIACT is a solid-phase two site immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote sites on the PSA molecule. The first one is coated on the solid phase (coated tube); the second, specific for free PSA and radiolabeled with iodine 125, is used as a tracer.

The free PSA molecules present in the standards or the samples to be tested are “sandwiched” between the two antibodies. Following the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step. The radioactivity bound to the tube is proportional to the concentration of free PSA present in the sample.

**4. REAGENTS**

Each kit contains enough reagents for 50 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
<b>COATED TUBES</b> : ready for use. Anti-PSA monoclonal antibody coated on the bottom of the tube.	1 pack of 50 tubes	2-8°C until the expiry date of the tracer. Unused coated tubes removed from their pack must be stored in the plastic bag supplied with the kit.
<b>ANTI free PSA 125 I</b> : ready for use. 125I anti free PSA monoclonal antibody, buffer, bovine serum albumin, sodium azide, red dye, non immunized mouse immunoglobulins. ≤ 185 kBq (≤ 5 µCi).	1 11 mL vial	2-8°C until the expiry date of the tracer.
<b>STANDARD 0</b> : ready for use. Human serum, sodium azide.	1 1 mL vial	2-8°C until the expiry date of the tracer.
<b>STANDARDS</b> : ready for use . Human free PSA, human serum, sodium azide, 0.5 - 1 - 2.5 - 5 - 20 ng/mL *.	5 0.5 mL vials	2-8°C until the expiry date of the tracer.
<b>CONTROL</b> : ready for use. Human free PSA**, human serum, sodium azide,	1 0.5 mL vial	2-8°C until the expiry date of the tracer.
<b>TWEEN 20</b> : Concentrated solution. Dilute 9 mL of Tween 20 in 3 L of distilled water. Shake gently.	1 10 mL vial	2-8°C until the expiry date. After dilution, keep in a capped container for a maximum of 15 days.
<b>PLASTIC BAG</b>	1	

(\*) The values shown above are target values only: the true value of each standard or control is shown on its label.

x ng CIS = 0.83 \* y ng 1<sup>st</sup> IS 96/668 (or 10 ng CIS = 12 ng 1<sup>st</sup> IS 96/668).

(\*\*) The acceptance range true values are printed on the vial label.

**After opening, all unused reagents should be stored under the conditions given above, until the expiry date printed on the kit.**

**5. PRECAUTIONS FOR USE**

**5.1. Safety measures**

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

## 5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

Enforcement of the basic radioprotection rules will ensure adequate safety.

A summary of these is given below:

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

## 5.3. Handling precautions

Do not use kit components beyond their expiry date. Do not mix reagents from different batches. Do not use more than 50 tubes at the same time.

Avoid any microbial contamination of the reagents or of the water. Fully respect the incubation times and the washing instructions indicated.

## 6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma without citrate (EDTA, heparin). If the test is to be carried out within 24 hours, serum and plasma must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots and deep frozen (-20°C) until needed.

### Dilutions

Given the clinical indication of free PSA assay, results should be read within the standard range proposed. However, dilutions may be carried out with the 0 standard supplied in the kit.

## 7. INTERFERENCES

No interferences with test results have been detected for concentrations of bilirubin < 0.5 mg/mL, of haemoglobin < 5 mg/mL, or triglycerides < 11 mg/mL.

## 8. ASSAY PROCEDURE

### 8.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 100 µL, 200 µL, 2 mL (± 1%). Their calibration should be checked regularly.

Distilled water. Disposable plastic tubes. Vortex-type mixer. Gamma scintillation counter calibrated for 125 iodine measurement.

### 8.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of the reagents into the tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes: Standard "0" group, for the determination of non specific binding. Standard groups, to establish the standard curve.

Control group for the control. Six groups, to test serum or plasma samples.

It is recommended that the assay be performed in triplicate for the standards and in duplicate for the samples.

Strictly observe the order in which reagents are to be added:

Dispense 100 µL of standards, control or samples to be assayed into the corresponding tubes.

Add 200 µL of <sup>125</sup>I anti free PSA monoclonal antibody to each tube.

Mix each tube gently with a Vortex-type mixer.

Incubate for 18 h ± 2 h at room temperature (18-25°C).

Wash the tubes as follows:

Aspirate the contents of the tubes as completely as possible.

Add 2.0 ml of washing solution to each tube, and re-aspirate.

Repeat the process once more.

Aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be performed correctly: the addition of the washing solution must be carried out with sufficient speed to create turbulence in the tubes.

Measure the remaining radioactivity bound to the tubes with a gamma scintillation counter.

## 9. QUALITY CONTROL

Good laboratory practices require that quality control samples 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.

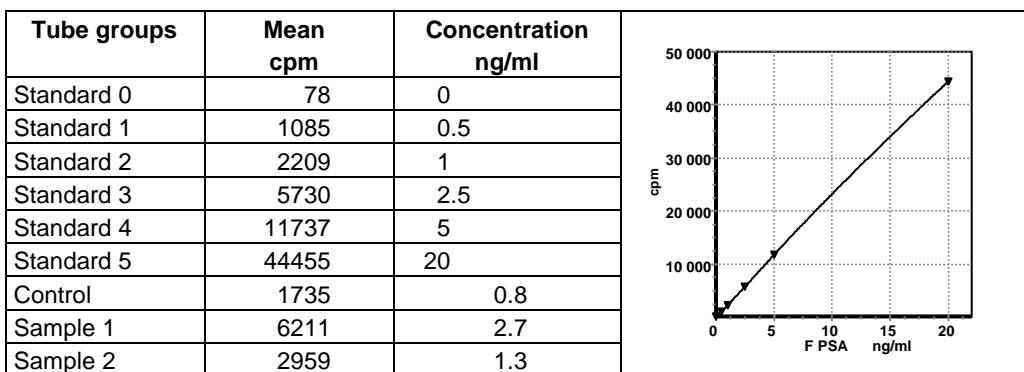
## 10. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.

Draw up the standard curve by plotting the standards' cpm against their concentrations.

Read the sample values directly from the curve.

**Typical standard curve** (example only): these data must not be substituted for results obtained in the laboratory.



**11. PROCEDURAL LIMITATIONS**

Samples with fibrin, gross haemolysis, gross lipemia or turbidity may give misleading results.

**12. EXPECTED VALUES**

Total PSA assayed must be taken into account in the interpretation of a free PSA assay. The result is shown as a percentage of free PSA over total PSA.

A preliminary study of 58 prostatic pathologies (cancers and benign hypertrophies) showed that the percentage of free PSA over total PSA may vary from 2 - 39% for values of total PSA ranging from 4 - 10 ng/mL.

**13. SPECIFIC CHARACTERISTICS OF THE ASSAY**

**13.1. Imprecision**

This has been assessed using 2 samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 10 different series.

Samples	Mean ng/mL	Within-run CV %	Between-run CV %
1	2.8	1.6	3.6
2	6.3	1.9	2.5

**13.2. Dilution test**

Ten samples with high levels were diluted, with the recovery percentages ranging from 90 to 110%.

**13.3. Specificity**

The antibody pair used in this assay guarantees specific measurement of free PSA. Interference from the PSA-ACT complex is less than 1%.

**13.4. Detection limits**

The detection limit measured by analytical method is defined as being the smallest detectable concentration different from zero with a probability of 95%. It has been assessed as being 0.02 ng/mL.

The functional detection limit is defined as being the measured concentration by imprecision profile for a CV equal to 20%. It has been assessed as being 0.16 ng/mL.

**13.5. Interference to HAMA (Anti-Mouse Human Antibodies)**

No interference to HAMA (measured up to 1500 ng/mL) has been observed.

**ASSAY FLOW CHART**

Tubes	Standard 0 $\mu$ L	Standards Control Serum or Plasma Samples $\mu$ L	$^{125}$ I anti-free PSA $\mu$ L	Mix gently Incubate 18 h $\pm$ 2 h at 18-25°C Wash twice	Count
Standard 0	100	--	200		
Standards	--	100	200		
Control	--	100	200		
Samples	--	100	200		