MARKIT-M PA

Enzyme-Linked ImmunoSorbent Assay（ELISA）

For the determination of total Prostate Specific Antigen (PSA) in plasma and serum

■Reagents

Each kit (96 tests) contains the following reagents.
Standard reagent 0 (lyophilized): 1 vial (for 0.5 mL).
Standard reagent 1 (lyophilized): 1 vial (for 0.5 mL) contains: PSA 0.5 ng
Standard reagent 5 (lyophilized): 1 vial (for 0.5 mL) contains: PSA 2.5 ng
Standard reagent 20 (lyophilized): 1 vial (for 0.5 mL) contains: PSA 10 ng
Standard reagent 50 (lyophilized): 1 vial (for 0.5 mL) contains: PSA 25 ng
Standard reagent 100 (lyophilized): 1 vial (for 0.5 mL) contains: PSA 50 ng
Labeled antibody (lyophilized): 1 bottle (for 15 mL)
Peroxidase-labeled anti-PSA monoclonal antibody (mouse)
Buffer solution : 1 bottle (15 mL)
Antibody-coated plate: 1 plate (96 wells)
Anti-PSA monoclonal antibody (mouse) coated wells
Substrate : 3 tablets
o-phenylenediamine dihydrochloride (OPD) (13 mg/tablet)
Solvent for substrate: 3 bottles (15 mL each)
Washing reagent concentrate: 3 bottle (30 mL)
Reaction stopping reagent: 1 bottle (15 mL) (ready-to-use)
Graph paper: 1 sheet.

■Intended Use

Quantitative determination of total PSA in human plasma and serum

■Principle of the assay

Two-step direct sandwich enzyme-linked immunosorbent assay (ELISA) using mouse monoclonal antibodies directed against two different epitopes of PSA.

■Procedure

1. Materials required but not provided

In addition to standard laboratory equipment, the following items are required:
Pipettes with disposable tips 25, 500 μL, Multichannel pipette 100 μL, ELISA washer, Microplate mixer, Microplate reader equipped with 492 nm (as the main

wave length) and 620 nm (as the reference wave length).

2. Specimen

Serum and plasma [acceptable anticoagulants: sodium heparin, potassium EDTA, and sodium EDTA] are acceptable specimens.

The separated samples may be stored frozen at -20°C for up to 3 months.

3. Preparation of reagents

(1) Reconstitution of Standard reagents

The content of Standard reagents is reconstituted with 0.5 mL of purified water. Wait at least 15 minutes and mix the vials gently to dissolve the content thoroughly. (Reconstituted standard solutions are stored at 2-8^{o}C or frozen for 1 month.)

(2) Reconstitution of Labeled antibody

The content of the vial is reconstituted with whole volume of Buffer solution. Mix the vial gently to dissolve the content thoroughly. (Reconstituted solution is stored at 2-8^{o}C for 1 month.)

(3) Antibody coated wells

Cut and remove the seal adhering to the required number of wells just prior to use. Then discard the preservation liquid in the wells. Turn the wells upside down and tap on a paper towel to remove the preservation liquid*. Ensure that the pouch containing any unused strips is completely resealed. The strips are stored at 2 - 10°C until expiry date of the kit.

*Note: Never dry the wells completely.

(4) Wash buffer

Prepare required amount of Wash buffer by diluting Washing reagent concentrate 1:10 with distilled or deionized water. Wash buffer is stored at 2-10^{o}C and used within 1 week of preparation.

(5) Substrate solution

Prepare Substrate solution just prior to use. Put one substrate tablet (OPD) into one bottle of Solvent for substrate, mix gently, and keep it protected from light.

4. Procedure

- Let all the reagents come to room temperature.
- It is recommended to perform the assay in duplicate.

(1) Place desired number of antibody coated wells prepared as described above in the well frame.

(2) Add 100 µL of Labeled antibody solution to each well.

(3) Add 25 µL of sample or standard solution to assay wells. Mix gently.

(4) Cover the plate with aluminum foil etc. Incubate the plate for one hour at room temperature.
(5) Aspirate the contents of each well. Wash each well four times with wash buffer (300 μL/well) and then completely aspirate the contents.
(6) Add 100 μL of Substrate solution to each well.
(7) Cover the plate with aluminum foil to avoid exposure to light. Incubate the plate for 30 minutes at room temperature.
(8) Add 100 μL of Reaction stopping reagent to each well. Mix gently.
(9) Read the absorbance at 492 nm (main wave length) and 620 nm (reference wave length) within 2 hrs.
(10) The concentrations of the PSA in samples are calculated by reference to the standard curve obtained from the absorbance of the standard solutions.

■Measurement range

PSA 0.5-100 ng/mL (from analytical sensitivity to highest calibrator)

■Precautions for use or handling

1. General Precautions

(1) The reagents are for Research Use Only.
(2) Do not mix the reagents from kits of different lots.

2. Safety precautions

PSA from human origin contained in Standard reagent, was tested and found negative for HIV-1, HIV-2, Hepatitis B and HCV. However, the reagents should be handled as if capable of transmitting disease.

■Storage method and expiry period

Storage : Store at 2-10°C
Expiry period: The expiration date is printed on the outer box.

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