Technical Manual

Human Prostate Specific Antigen ELISA Kit

- Catalogue Code: HDES0112
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This kit uses double antibody sandwich method. The sample (calibrator or serum to be tested) was added into the coated PSA antibody microporous strip, and the enzyme-labeled antibody was added at the same time to specifically form a solid phase antibody-antigen-enzyme-labeled antibody complex. After the substrate was added for color development, OD values of each hole were determined by enzyme-labeled meter, and the sample content was calculated according to the calibration curve.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0，1，2，8，16，32 ng/mL)
HRP Conjugate	6 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash	15 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized or distilled water
- Absorbent paper

4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.

3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0112. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0112 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum** was quickly separated after blood collection to avoid hemolysis. Serum samples can be stored at 2~8° C for a week, long-term storage should be packaged, sealed and frozen below -15° C, can be stored for three months, and avoid repeated freezing and thawing (no more than 5 times).
2. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. Remove the pre-packaged plate from the sealing bag, set two wells for each standard product, and add the corresponding **Standard Liquid** 100μL to each hole; Add 100μL of **Serum** directly to each assay hole.
2. Then add 50μL of **HRP Conjugate** to each well, mix thoroughly, apply a sealing plate membrane, and incubate at 37 ° C for 1 hour.

3. Remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 3 times with **Wash Buffer** and immerse for 30-60 sec each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. Add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. incubate at 37° C for 15 min in shading light.
5. Add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
6. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection.

8. Result analysis

Using the linear fitting function, the concentration of each calibration product is logarithm (Log(concentration)) as X, the corresponding absorption value is logarithm (Log(OD)) as Y, and the double-logarithm (or full logarithm) log-log fitting equation is selected: Log(OD) = BLog(concentration) +A, and the concentration of the serum to be measured is calculated from the fitting line.

9. Limitations of test method

Rheumatoid factors, anti-animal antibodies and other factors that affect the immune response can affect the determination.

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Email: info@assaygenie.com

Web: www.assayenie.com