Human papillomavirus antibody (IgG) ELISA Kit

Catalog No. CSB-E08782h

(96T)

- This immunoassay kit allows for the in vitro semi-quantitative determination of **human papillomavirus antibody (IgG)** concentrations in **serum**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with human papillomavirus antigen. Sample Diluent and Samples are then added to the appropriate microtiter plate wells and incubated. Then add Horseradish Peroxidase (HRP)-conjugated anti-human IgG and incubated. Then substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of human papillomavirus antibody (IgG) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

SPECIFICITY

This assay recognizes human papillomavirus antibody (IgG) ,No significant cross-reactivity or interference was observed.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
HRP-conjugate	1 x 12 ml
Positive Control	1 x 500 µl
Negative Control	1 x 500 µl
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Wash Buffer	1 x 20 ml (20×concentrate)
Sample Diluent	3x 16 ml
Stop Solution	1 x 6 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 400 ml of Wash Buffer.
2. **Sample** Serum or Plasma samples require a 100-fold dilution into Sample Diluent. The suggested 100-fold dilution can be achieved by adding 10µl sample to 40µl of Sample Diluent. Complete the 100-fold dilution by adding 10µl of this solution to 190µl of Sample Diluent.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, and controls be assayed in duplicate.

1. Dilute the sample using Sample Diluent(1:100).
2. Add 100µl of Sample Diluent to Blank well. Add 100µl of Negative Control, Positive Control or diluted Sample per well. Incubate for 30 min at 37°C.

3. Aspirate each well and wash, repeating the process five times for a total of five washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100µl of **HRP-conjugate** to each well. Cover the microtiter plate with adhesive strip. Incubate for 30 min at 37°C.
5. Wash five times as before.
6. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 10 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
7. Add 50µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

For calculation the valence of human papillomavirus antibody (IgG), compare the sample well with control.

If $OD_{negative} < 0.1$, caculate it as 0.1
While $OD_{sample} / OD_{negative} \geq 2.1$: Positive
While $OD_{sample} / OD_{negative} < 2.1$: Negative

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.