NOVATEINBIO
Accelerates Research and Development

Human Papilloma Virus Type 6 IgG, HPV 6 IgG ELISA Kit

Catalog number: NB-E20758 (96 wells)

The kit is designed to qualitatively detect HPV 6 IgG in human serum or plasma.

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid from bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that the test samples are tested in duplicate.
- If the blue color develops too shallow after 10 minutes incubation with the substrates, it may be appropriate to extend the incubation time (Do not over develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.
- Chromogenic Solution B is light-sensitive. Avoid prolonged exposure to the light.

Intended use

This kit is used to qualitatively detect HPV 6 IgG in human serum or plasma.

Assay time	90 min
Validity	Six months
Store at	2-8 °C

Assay principle

This assay employs the enzyme-linked immunosorbent assay (ELISA) technology to detect HPV 6 IgG in the samples. Purified HPV 6 antigens are precoated in microplate wells. The anti-HPV 6 IgG in the samples, if presents, will bind to the HPV 6 antigen immobilized on the wells and interacts with anti-Human IgG antibody-HRP conjugate to form an immunocomplex. Following incubation and washing procedures to remove unbound substances, this reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). After stopping the reaction with sulfuric acid, the blue color turns yellow. What can be measured at this point is the amount of color intensity proportional to the amount of antibody captured in the wells, and to the sample.

Materials supplied

1	Microplate precoated with HPV 6 antigens	1x96 well
2	Negative Control	1 vial
3	Positive Control	1 vial
4	Diluent buffer	13 ml x1
5	Wash Solution (20x)	25 ml x1
6	Anti-Human IgG-HRP Conjugate	13 ml x1
7	Chromogenic Solution A	6 ml
8	Chromogenic Solution B (TMB)	6 ml
9	Stop Solution	6 ml
10	Closure plate membrane	2
11	Sealed bag	1
12	Package insert	1

Materials required but not supplied

- 37°C incubator.
- Standard microplate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 3000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

  NOTE:
- Samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 2 months) or -80°C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.
- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- **Wash Solution** - Dilute 25 mL of Wash Solution concentrate (20×) with 475 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×).

Assay procedures

1. Prepare all the controls and samples before starting assay procedure (Please read Reagents Preparation). It is recommended that samples should be added in duplicate to the Microtiter Plate.
2. Set one blank control well (neither samples nor HRP-Conjugate should be added into the blank well), three Negative control wells, two Positive control wells, and test sample wells on the assay plate. Add 100 µl Diluent buffer into the blank control well. Dilute Positive control, Negative control, and samples 1:20 with Diluent buffer. Add 100 µl of diluted Positive control, Negative control, and samples into their respective wells. Mix well. Cover and incubate the plate for 30 minutes at 37°C.
3. Wash the Microtiter Plate using one of the specified methods indicated below:
   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.
   Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
   Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µl/ well /wash) using an auto washer. After washing, dry the plate as above.It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
4. Add 100 µl HRP-conjugated antibody to each well. Mix well. Mixing well in this step is important. Cover and incubate the plate for 30 minutes at 37°C.
5. Discard the solution and wash the plate as step 3.
6. Add 50 µl Chromogenic Substrate A and 50µl Chromogenic Substrate B to each well, subsequently. Cover and incubate for 5-15 minutes at room temperature (Protect from light. Do not over develop).
7. Add 50 µl Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Determine the Result

- **Calculate the cut-off value (COV):**
  COV = the average OD value of Negative Control wells (NCOD450) + 0.12
  If the OD value of Negative Control wells (NCOD450) is less than 0.02, the OD value should be counted as 0.02; if the OD value of Negative Control wells (NCOD450) is more than 0.02, the actual OD value should be counted.

Note: If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

- **Quality control range:**

  The test results are valid if the Quality Control criteria are verified.

  1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.

  2. The OD value of the Positive control must be equal to or greater than 0.800 at 450nm after blanking.

  The OD value of the Negative control must be less than 0.100 at 450nm after blanking.

- **Interpretations of the results:**

  (S = the individual optical density (OD) of each specimen)

  Positive Results (S/COV≥1): samples giving an optical density greater than, or equal to the Cut-off value are considered initially reactive, which indicates that antibodies to HPV 6 have probably been detected using this anti-HPV 6 ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HPV 6 and therefore the patient is probably infected with hepatitis C virus.

  Negative Results (S/COV <1): samples giving an optical density less than the Cut-off value are considered initially non-reactive, which indicates that antibodies to HPV 6 have probably not been detected using this anti- HPV 6 ELISA kit. Retesting in duplicates of non- reactive sample is encouraged.

  Borderline: Samples with optical density O.D.≤ COV ×2 are considered borderline and retesting of those samples in duplicates is recommended. Repeatedly positive samples could be considered positive for HPV infection.

Manufactured and Distributed by:
Novatein Biosciences
310 West Cummings Park

Woburn, MA, 01801, USA
Phone: (617) 238-1396
Fax: (617) 380-0053
Toll Free: (888) 856-2858

http://www.novateinbio.com/
Email: Info@novateinbio.com