INTENDED USE

The **Human Anti-HPVL1 IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for **HPV subtype 6, 11, 16, and 18** L1 proteins in a single sample of serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.
This immunoassay is suitable for:

- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols

The assay is for research use only (RUO) and is not intended nor validated for diagnosing HPV. Reagents contain no virus.

GENERAL INFORMATION

Human papillomavirus (HPV) causes cervical cancer, the third most common cancer in women worldwide. Lifetime incidence of HPV infection is estimated to be 80%. Like all papillomaviruses, HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. Most infections become undetectable within 1–2 years and only a small fraction of infections with high-risk HPV fail to clear, resulting in overt HPV persistence. Over 120 HPV types have been identified and are referred to by number;types16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are "high-risk" sexually transmitted HPVs. Neutralizing antibodies are expected to be the primary immune mechanism for protection against infection.

Two **vaccines** are available to prevent infection by some HPV types: **Gardasil (**Merck) and **Cervarix (GSK)**. Both vaccines utilize recombinant L1 proteins and protect against initial infection with HPV types.

PRINCIPLE OF THE TEST

The Human Anti-HPV IgG ELISA kit is based on the binding of anti-HPV IgG in samples to HPV antigens immobilized on the microwells, and anti-HPVIgG antibody is detected by anti- IgG-specific antibody-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-HPV IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA reader. The presence of human IgG antibody in samples is determined relative to anti-HPV Calibrators.

PRODUCT SPECIFICATIONS

Specificity
A mixture of purified recombinant (*E. coli*) HPVL1 proteins (6+11+16+18) is used as antigens; thus, this test detects all 4 HPV antibodies in a single test. HPVL1's from HPV6, 11, 16, and 18 subtypes share ~50% sequence homology. The Anti-human IgG HRP conjugate reacts specifically with human IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals.

Assay Sensitivity
The HPVL1 antigen coating level and HRP conjugate concentration are optimized to differentiate anti-HPV IgG from background (non-antibody) signal with human serum or plasma samples diluted 1:100.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4°C for long term and ambient temp.for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.211, 0.15ml	Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
HPVL1 6, 11, 16, 18 Coated Strip Plate	550-101	8-well strips (12)	Coated with HPVL1 6+11+16+18 subtypes recombinant proteins, and post-coated with stabilizers.
Anti-HPV Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	550102B 550102C 550102D 550102E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-HPVL1; in buffer with antimicrobial as stabilizers.
Anti-HPV Positive Control	550-102PC	0.65 ml	Serum with anti-HPVL1reactivity; Net OD >**0.5**
Low NSB Sample Diluent	TBTm **Not** for HRP dilution	30 ml	Buffer with protein, detergents and anti-microbial. Use as is for sample dilution
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgG HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.
Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Antibody Stability& Dilution

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.
Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design
Review Interpretation of Results and Limits of the Assay (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually **1/100** or greater dilution for human serum/plasma with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the Anti-HPV **Positive Control;** net OD >**0.5**.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up
Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

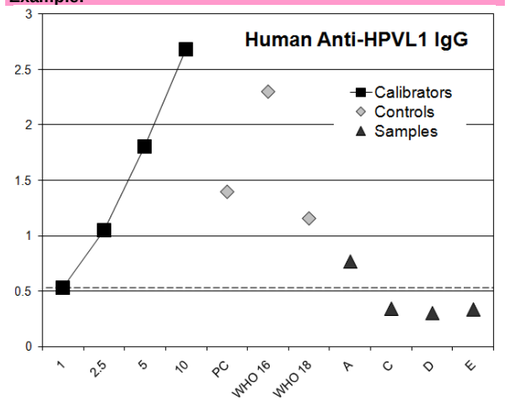
INTERPRETATION OF RESULTS

Method A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

=Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-HPV IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-HPV antibody, derived from HPVL1 vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – an antiserum with reactivity to HPVL1 antigens; net OD >**0.5**. This Control can be used to normalize between-assay variation.

WHO International Standards for **HPV16** [NIBSC 05/134] and **HPV18** [NIBSC 10/140]:
HPV16 – diluted to**1.0** IU/ml = **7.4** U/ml in the assay.
HPV18 – diluted to **1.6** IU/ml = **2.75** U/ml in the assay.
WHO standards also reacted with HPV6 and 11 but no units were assigned by WHO.

Samples A,B,C,D – 3 samples (B, C, D) are negative: below the threshold; 1 sample (A) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Method B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.

2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Example:

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.244	**C** 2.293	0.57	**5.34**
2	0.204	**C** 1.490	0.48	**3.47**
3	0.237	**C** 0.833	0.55	**1.94**
4	0.26	**C** 0.326	0.61	0.76
5	0.388	**P** 1.106	0.90	**2.58**
6	0.407	**I** 0.310	0.95	0.72
7	0.288	**E** 0.672	0.67	**1.56**
8	0.263	**E** 0.363	0.61	0.85
9	0.322	**E** 0.560	0.75	**1.31**
10	0.343	**E** 0.490	0.80	1.14
Mean	0.295			
SD	0.067			
Mean +2 SD	**0.429**	= **Positive Index**		

Results

Experimental Samples are represented as follows:

C – Calibrator
P – Positive Control
I – Internal Control; lab's threshold positive serum
E – Experimental sample

ASSAY PERFORMANCE

Method C. Titers from Sample Dilution Curves
The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Positive and Sensitivity Control values can be used to normalize inter-assay values.

Calculations
1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index
   = **IgG Antibody Activity Units**

Notes:
- The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a **Positive Index** (page 6), or by using known **Internal Controls** as discriminator for a **Threshold Control** (instead of the kit 1 U/ml Calibrator Control)

Limits of the Assay
- The assay detects and quantifies IgG antibodies directed to the L1 protein. Patients may have HPV infection without producing antibodies specific to L1.
- Anti-HPV antibody levels of an infected patient may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.
- Samples from non-patients may be elevated due to prior exposure to the human papilloma virus.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website:http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

Instruction Manual No. M-550-100-PHG

Human Anti-HPVL1 [6+11+16+18 Late Protein] IgG ELISA Kit

Cat. No.550-100-PHG

For the Detection and Quantitation of Anti-HPVL1 IgG in Human Serum or Plasma

For research use only, not for diagnostic or therapeutic use.

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