# RayBio® Preliminary Human Papillomavirus (HPV) Human ELISA Kit

User Manual Version 1.0
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Catalog numbers: IEQ-HPV-PRELIM-1 (1 plate)
IEQ-HPV-PRELIM-2 (2 plates)
IEQ-HPV-PRELIM-5 (5 plates)



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Empowering your proteomics

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RayBiotech, Inc.

# RayBio® Human Papillomavirus (HPV) Human ELISA Kit

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Please read the entire manual carefully before starting your experiment

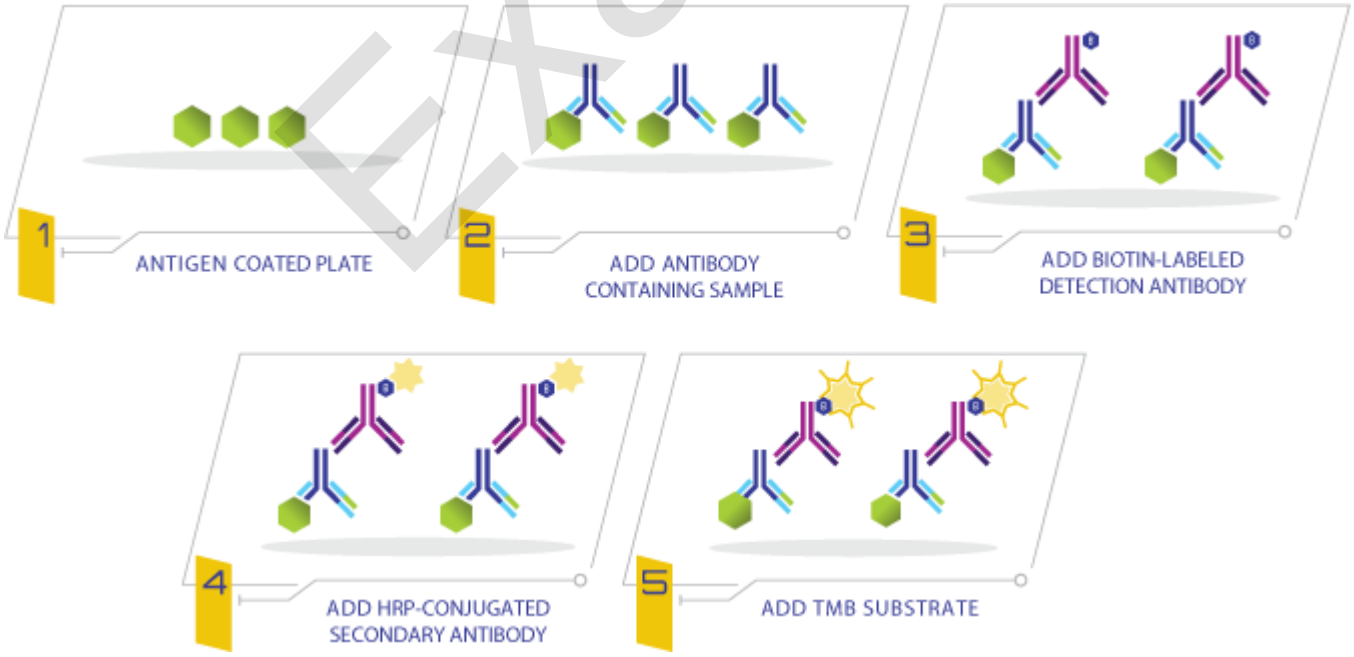
# I. INTRODUCTION

The Preliminary Human Papillomavirus (HPV) Human ELISA Kit is an in vitro indirect ELISA for the semi-quantitative measurement of human IgG or IgM (IgG/M) antibody against HPV antigen(s) in human serum or plasma. This ELISA kit is for research use only, not for therapeutic or diagnostic applications.

PRINCIPLE OF THE ASSAY

This HPV human antibody ELISA kit employs an indirect ELISA method. In this kit, standard 96-well plates (12 strips with 8 wells/strip) are coated with a HPV protein, which combines with the corresponding antibody present in a sample and Positive Control, which is used as a calibration curve for interpretation purposes. When a biotinylated secondary anti-human IgG/M antibody is added, a complex forms between biotinylated secondary antibody and human antibody bound to the protein coated plate. A TMB substrate is added, and a blue color is generated. The depth of color is relative to the amount of the human anti-HPV antigen antibody present. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

The Positive Control consists of an anti-HPV antigen antibody. A biotinylated anti-Positive Control antibody is added to the Positive Control wells and treated identically to the samples. The intensity of the resulting color (measured at 450 nm) is used as a calibration curve for interpretation purposes in the different assays.



## II. STORAGE

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

## III. REAGENTS

| **Component** | **Size / Description** | **Storage / Stability <u>After Preparation</u>** |
|---|---|---|
| HPV protein coated 96-well Microplate | 96 wells (12 strips x 8 wells) coated with HPV protein | 1 month at 4°C* |
| Wash Buffer Concentrate (20X) (Item B) | 25 ml of 20X concentrated solution. | 1 month at 4°C |
| Positive Control (Item C) | 1 vial of Positive Control sample containing anti-HPV antigen antibody | 1 week at -80°C |
| Biotinylated Anti-Human IgG/M (Item F) | 1 vial of solution. | 5 days at 4°C |
| Biotinylated Anti-Positive Control (Item F-2) | 1 vial of solution. | 5 days at 4°C |
| HRP-Streptavidin concentrate (Item G) | 1 vial of solution. | Do not store and reuse |
| TMB One-Step Substrate Reagent (Item H) | 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB buffer solution). | 1 month at 4°C |
| Stop Solution (Item I) | 8 ml of 0.2 M sulfuric acid. | N/A |
| Assay Diluent B (Item E) | 15 ml of 5X concentrated buffer. | 1 month at 4°C |
| 5X Sample Diluent (Item J) | 25 ml of 5X diluent buffer, 0.5% proclin 300 as preservative. | 1 month at 4°C |

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

## IV. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.

# V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. 5X Sample Diluent (Item J) should be diluted 5-fold with deionized or distilled water before use to make 1X Sample Diluent.

3. 5X Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use to make 1X Assay Diluent B.

4. Dilute sample (human serum) with 1X Sample Diluent (Item J) 1500 times. For example, add 1 µl serum + 1499 µl 1X Sample Diluent. Mix the diluted sample well and evenly for the best results.

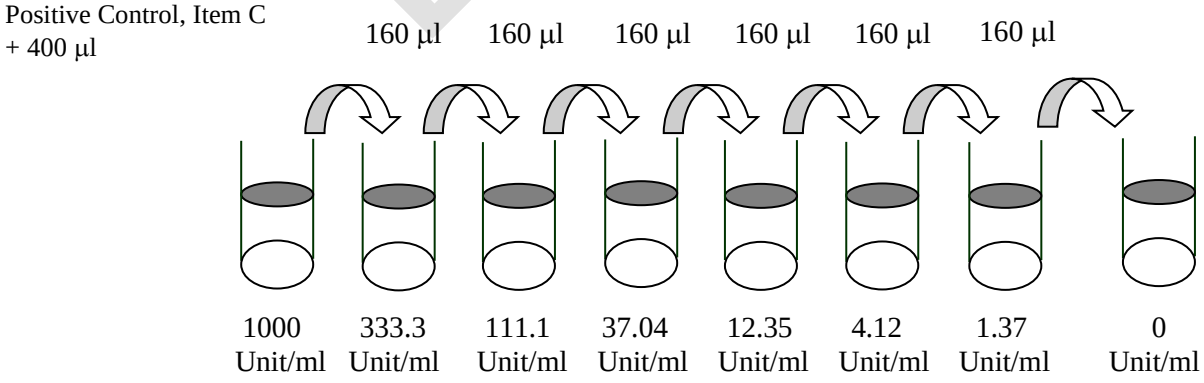
   Note 1: The user needs to calculate the amount of the sample used for the whole test. Please reserve sufficient amount of sample in advance.

   Note 2. Avoid using samples with severe hemolysis, precipitate, contamination by bacteria or protein suspension.

   Note 3. The use of EDTA, heparin sulfate, sodium citrate, or other anticoagulants will not affect the results.

5 . Preparation of Positive Control calibration curve: **Briefly spin the vial of Positive Control, Item C**. Add 400 µl 1X Sample Diluent (Item J) into the Item C vial to prepare a 1000 Unit/ml Positive Control solution and mix thoroughly. Pipette 320 µl 1X Sample Diluent into each of 7 tubes. Use the 1000 Unit/ml Positive Control solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Sample Diluent serves as the zero (0 Unit/ml).





6. If the Wash Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

7. Briefly spin the Biotinylated Anti-Positive Control Antibody vial (Item F-2) before use. Add 200 µl of 1X Assay Diluent B (Item E) into the vial to prepare an antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B and used in step 5 of Part VI Assay Procedure.

8. Briefly spin the Biotinylated Anti-Human IgG/M Antibody vial (Item F) before use. Add 200 µl of 1X Assay Diluent B (Item E) into the vial to prepare an antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B and used in step 5 of Part VI Assay Procedure.

9. Briefly spin the HRP-Streptavidin concentrate (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 800-fold with 1X Assay Diluent B (Item E) and used in step 7 of Part VI Assay Procedure.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 12.5 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent B to prepare a 800-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

## VI. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that the positive control, and all samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each prepared positive control (Item C, prepared in Reagent Preparation step 5), and sample (prepared in Reagent Preparation step 4) into appropriate wells. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µl of 1X Wash Buffer using a multi-channel Pipette or autowasher. Complete removal of all liquid at each step is essential for 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.

5. Add 100 µl of prepared Biotinylated Anti-Positive Control Antibody (Item F-2, Reagent Preparation step 7) and Biotinylated Anti-Human IgG/M Antibody (Item F, Reagent Preparation step 8) to each of the Positive Control and sample wells, respectively. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 9) to each well. Incubate for 30 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

10. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

11. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

## VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl positive control, or sample to each well. Incubate 1 hour at room temperature.

3. Add 100 µl prepared Biotinylated Anti-Positive Control and Biotinylated Anti-Human IgG/M Antibody into respective wells. Incubate 1 hour at room temperature.

4. Add 100 µl prepared HRP-Streptavidin solution to each well. Incubate 30 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

## VIII. INTERPRETATION OF RESULTS

1. Calibration curve: Calculate the mean absorbance for each set of duplicate Positive Control (Item C), and samples, and subtract the average zero Positive Control optical density. Plot the calibration curve on a log-log scale with Positive Control concentration (Unit/ml) on the x-axis and absorbance on the y-axis using Excel or statistical software.

## IX. ASSAY PERFORMACE

# X. TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|---|---|
| Low signal | Improper preparation of positive control and/or antibodies | Briefly spin down vials before opening. |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation. |
| | Insufficient incubation times | Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). |
| Large CV | Inaccurate pipetting | Check pipettes. |
| | Air bubbles in wells | Remove bubbles in wells. |
| High background | Plate is insufficiently washed | Review the manual for proper wash procedure. If using a plate washer, ensure that all ports are unobstructed. |
| | Contaminated wash buffer | Make fresh wash buffer. |

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