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HPV16 E7/Protein E7 ELISA Kit

Catalog No: KVV08902 **Quantity:** 1 plate **Lot:** xxxxx **Expiration:** 6+ months

Specificity:	HPV16 Protein E7
Sensitivity:	37.98 pg/ml, minimum detectable dose (MDD) HPV16 E7
Range:	93.75 – 6,000 pg/mL
Sample Type:	Serum and plasma

MATERIALS PROVIDED & STORAGE CONDITIONS

PART	FORMAT	DESCRIPTION	STORAGE
Pre-coated Microplate	1 plate	96-well microplate (12 strips of 8 wells) precoated with antibody specific for HPV16 E7.	Store sealed at -20 °C
HPV16 E7 Standard	2 bottles	6,000 pg/bottle of lyophilized HPV16 E7. Reconstitute with 1 mL Standard Diluent before use.	Store at -20 °C
Detection A	1 vial	120 µL/vial of Biotin labeled antibody specific for HPV16 E7, 1:100 diluted by Assay Diluent before use	Store at -20 °C
Detection B	1 vial	120 µL/vial of Streptavidin-HRP (including, 1:100 diluted by Assay Diluent before use.	Store at -20 °C
Standard Diluent	1 bottle	25 mL/bottle diluent for use to dilute the Standard and Samples.	Store at 2 - 8 °C
Assay Diluent	1 bottle	25 mL/bottle diluent for use to dilute the Detection A and Detection B.	Store at 2 - 8 °C
20 × Wash Buffer	1 bottle	25 mL/bottle of 20X solution of buffered surfactant with preservative, 1:20 diluted with deionized water before use.	Store at 2 - 8 °C
Color Reagent	1 bottle	12 mL/bottle of TMB (Tetramethylbenzidine).	Store at 2 - 8 °C
Stop Solution	1 bottle	6 mL/bottle.	Store at 2 – 8°C
Plate Sealers	4 strips	Adhesive strips.	Store at RT.

Note: Store unopened kits at 2-8°C upon receipt.
To prolong storage life when opened, store the Standard, Detection A, Detection B and Microplate at -20 °C, and the remaining reagents at 2-8°C.

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SARS-CoV-2 RBD (KP.2) ELISA Kit

INTENDED USE

Used for the quantitative determination of HPV16 E7 concentration in serum and plasma.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for HPV16 E7 has been pre-coated onto a microplate. Standards or samples are pipetted into the wells and any HPV16 E7 present binds to the immobilized antibody. After washing away any unbound substances, a biotin-labeled antibody specific for HPV16 E7 is added to the wells. After washing away any unbound substances, Streptavidin-HRP is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HPV16 E7 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 620 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- 500 mL graduated cylinder.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Handle all blood and serum as if capable of transmitting infectious agents. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

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

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq$ -20 °C. Avoid repeated freeze-thaw cycles.

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REAGENT PREPARATION

Bring all reagents to room temperature before use.

20X Wash Buffer Concentrate - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of **Wash Buffer**.

Serum and Plasma - Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 10 µL of sample + 90 µL of Standard Diluent (diluted 1:9). If the sample value is outside the range of the standard curve, the dilution can be adjusted appropriately, and the assay can be redetermined. If the antibody concentration in the sample can be estimated and the assay can be performed simultaneously by diluting several gradients prior to the experiment.

Standard - Reconstitute **Standard Diluent** with 1 mL, this reconstitution produces a **stock solution** of 6,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. 6,000 pg/mL is the first standard point, and the concentration of the 7 standard samples are 6,000 pg/mL, 3,000 pg/mL, 1,500 pg/mL, 750 pg/mL, 375 pg/mL, 187.5 pg/mL, 93.75 pg/mL respectively. The appropriate **Standard Diluent** serves as the zero standard (0 pg/mL).

Note: Reconstitute a new Standard vial for each assay and discard after use. Avoid freeze/thaw cycles.

Detection A (working solution) – Shake gently to mix before use. **Centrifuge briefly with palm centrifuge to consolidate the liquid at the bottom of the tube.** Dilute the **Detection A** 1: 100 with **Assay Diluent** for the working concentration **immediately before use. Only use the volume of stock solution needed for the assay.**

Detection B (working solution) - Shake and mix before used. **Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube.** Dilute the **Detection B** 1: 100 with **Assay Diluent** for the working concentration **immediately before use. Only use the volume of stock solution needed for the assay.**

Note：Please perform simple centrifugation to collect Detection A and Detection B before use.

Prepare Detection A and Detection B immediately before use and avoid diluting the entire stock at once.

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ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of standards or samples to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C.
4. Aspirate each well and wash, repeating the process three times. Wash by filling each well with **Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or auto-washer. 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 on clean paper towels.
5. Add 100 µL of **Detection A** (working solution) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C.
6. Repeat the aspiration/wash as in step 4
7. Add 100 µL of **Detection B** (working solution) to each well. Cover with a new adhesive strip. Incubate for 30 minutes at 37°C.
8. Aspirate each well and wash, repeating the process **FIVE** times. Wash by filling each well with **Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or auto-washer. 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 on clean paper towels.
9. Add 100 µL of **Color Reagent** to each well. Incubate for 15 minutes at 37 °C. Protect from light.
10. Add 50 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm or 620 nm. If wavelength correction is not available, subtract readings at 630 nm or 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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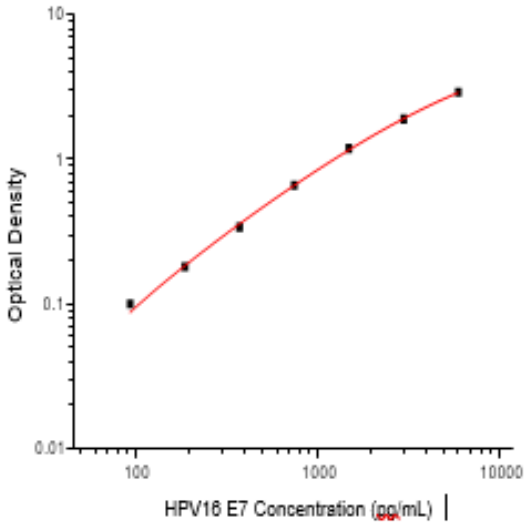
CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample. Construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the HPV16 E7 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.



(pg/mL)	O.D.	Average	Corrected
0	0.02	0.02	-
	0.02		
93.75	0.12	0.12	0.10
	0.11		
187.5	0.21	0.20	0.18
	0.18		
375	0.37	0.36	0.34
	0.34		
750	0.70	0.68	0.66
	0.66		
1500	1.26	1.21	1.19
	1.16		
3000	1.98	1.92	1.90
	1.86		
6000	2.97	2.93	2.91
	2.89		

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

ASSAY RANGE

93.75 pg/ml – 6,000 pg/ml

SENSITIVITY

The minimum detectable dose (MDD) of HPV16 E7 is typically less than 37.98 pg/ml.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero-standard replicates and calculating the corresponding concentration.

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PRECISION

Intra-Assay Precision (Precision within an assay): <10%
Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision.
Inter-Assay Precision (Precision between assays): <15%
Three samples of known concentration were tested in twenty-four separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	1	24	24	24
Mean (pg/mL)	2412.7	682.0	184.5	2417.0	633.3	158.8
Standard deviation	187.6	42.2	14.0	211.4	27.4	19.8
CV (%)	7.8	6.2	7	8.7	4.3	12.5

STABILITY

When the kit was stored at the recommended temperature for 6 months, the signal intensity decreased by less than 20%.

RECOVERY

The recovery of HPV16 E7 spiked to three different levels in human serum samples throughout the range of the assay was evaluated.

Sample	Dilution	Average	Range
Human serum (n=3)	1:10	95	83-107
	1:20	93	85-101

LINEARITY

To assess the linearity of the assay, human serum samples were spiked with high concentrations of HPV16 E7 and diluted with the **Standard Diluent** to produce samples with values within the dynamic range of the assay. (Human serum samples were prediluted 10-fold.)

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Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=3)	1:2	103	102-105
	1:4	104	99-108
	1:8	110	107-118
	1:16	106	99-120

TROUBLESHOOTING GUIDE

Problem	Probable Cause	S o
Poor Precision	Wells are not washed or aspirated	Make sure the wash apparatus works properly, and wells are dry after aspiration
	Bubbles in the wells	Tap plate gently to disperse bubbles
	Wells are scratched with pipette tip or washing	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
High background	Plate is not washed properly	Make sure the wash apparatus works properly
	Incorrect incubation times and/or temperatures	The OD value increased gradually with time. Reduce the color developing time properly
Weak/No Signal	Pipetting errors	Make sure the pipette is calibrated
	The working solution not prepared immediately before use	The working solution should be prepared immediately before use and should not be stored
	Volumes errors	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	Detection A working solution is not completely mixed with the samples	After adding **the Detection A** to the wells, make sure the samples are mixed thoroughly

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