Vaccinia Antibody Detection ELISA

Cat. #: VLET-001

Determining Vaccinia antibody titers is useful in population screening for monitoring antibody levels, and as a research tool for determining cowpox vaccine efficacy. Vaccinia (Lister strain) has been used by researchers to follow overall antibody titers. On serum drawn from vaccinated individuals, the Vaccinia ELISA has a greater than 95% correlation.

REAGENTS PROVIDED TO PERFORM 92 TESTS

- One (1) Vaccinia Lister heat-inactivated antigen-coated plate
- 4.0 µl Vaccinia Positive Control Serum
- 4.0 µl Negative Control Serum
- 10 ml Goat anti-Human IgG (H+L) Peroxidase Conjugate Solution - ready-to-use
- 25 ml Sample Dilution Buffer - ready-to-use
- 10 ml ABTS Substrate Solution - ready-to-use
- 10 ml Stop Solution - ready-to-use
- 30 ml 20X Wash Solution (dilute 1:20 with laboratory high-purity-grade water)

NOTE: Store all provided kit reagents at 2-8°C.

EQUIPMENT AND MATERIALS REQUIRED

a) High precision pipette (i.e., 1-20 microliter pipette)
b) 0.2 ml, 1.0 ml and 5.0 ml pipettes
c) 8 or 12 channel pipette (or transfer pipette)
d) 2 graduated cylinders (50 ml)
e) 1 ml or 5 ml borosilicate glass test tubes or plastic tubes
f) Uncoated low binding 96 well plates (i.e., Nunc catalog #269620)
g) Laboratory grade (Distilled or R.O. deionized) water
h) 96-well plate reading spectrophotometer with 630 nm filter and 492 nm differential filter
i) Plate washing apparatus

PRECAUTIONS

a) Handle all reagents and samples as biohazardous material.
b) Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
c) Wash solution, control sera, test plates, field samples and all other test reagents should be properly decontaminated with bleach or autoclaving before disposal.
d) Take special care not to contaminate any of the test reagents with serum or bacterial agents.
e) The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
f) Do not use this kit after the expiration date.

NEVER PIPETTE BY MOUTH.

ALLOW ALL REAGENTS TO COME TO ROOM TEMPERATURE BEFORE STARTING!

SAMPLE COLLECTION

For routine serologic population monitoring, it is suggested that at least 30 or more sera per population be randomly collected from different age groups. Proper sample collection procedures, serum harvest and serum sample storage (4°C for up to four days or -20°C for longer periods) are needed to provide reliable test results.

SAMPLE DILUTION PROCEDURE

Dilute serum samples, positive and negative controls 1:100 using Dilution Buffer in a clean, uncoated 96-well microtiter plate (serum dilution plate). Set up samples and controls such that wells A1 and A2 contain the Negative Control Serum and wells A3 and A4 contain the Positive Control Serum. The remaining wells are to be used for the test samples.

Please Note: Diluted serum should be tested within eight (8) hours.

PREPARATION OF THE SERUM DILUTION PLATE

a) Add 200 µl Dilution Buffer to each well of uncoated 96-well microtiter plate, the serum dilution plate.
b) Add 2 µl each of negative control in wells A1 and A2 and 2 µl positive control in wells A3 and A4.

c) Add 2 µl diluted unknown serum per well (producing a 1:100 dilution). Start with well A5 and end with well H12 (moving left to right, row by row of wells).

**Please Note:** Allow all diluted serums to equilibrate in Dilution Buffer for 5 minutes on a rotary shaker set at 100 rpms before transferring to a VACCINIA antigen

**Preparation of 1X Wash Solution (1:20)**
Dilute 20 ml concentrated Wash Solution in 380 ml laboratory grade (distilled or R.O. deionized) water. Mix well. Approximately 500 ml Wash Solution is needed for each 96-well ELISA plate.

**Preparation of Substrate Solution**
The Substrate Solution is ready to use. Each plate will require approximately 10 ml Substrate Solution. For best results, the Substrate Solution must be equilibrated to room temperature before use.

**Preparation of Enzyme Conjugate**
The Enzyme Conjugate is ready to use. Allow the enzyme conjugate to equilibrate to room temperature before use. Swirl bottle to mix.

**Preparation of Stop Solution**
The Stop Solution is ready to use. Allow Stop Solution to equilibrate to room temperature before use. Swirl bottle to mix.

**ELISA TEST PROCEDURE**

**PREPARING THE TEST PLATE**

a) Remove a VACCINIA antigen coated test plate from the protective bag and label according to dilution plate identification.

b) Add 50 µl Dilution Buffer to all wells on the test plate.

c) Add 50 µl diluted negative controls to wells A1 and A2. Add 50 µl diluted VACCINIA Positive Control Serum to wells A3 and A4. Discard pipette tip. Using an 8 or 12-channel pipette transfer 50 µl/well of each of the diluted serum samples and Normal Control Serum samples from the dilution plate to the corresponding wells of the VACCINIA coated test plate; mix up and down with pipettor to equilibrate samples. Discard pipette tips after each row of sample is transferred. Transfer of samples to the ELISA plate should be done as quickly as possible. Tap the plate gently to mix.

d) Incubate the plate for 120 minutes at room temperature.

**WASH PROCEDURE**

e) Tap out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.

f) Using an 8 or 12 channel pipette (or comparable automatic washing device), fill each well with approximately 300 µl Wash Solution. Allow soaking in wells for ten seconds; then discard contents into an appropriate waste container (waste container should contain bleach solution). Tap inverted plate to ensure that all residual liquid is removed. Repeat wash procedure three more times.

**Please Note:** The wash procedure is a very critical step in any ELISA procedure. Please follow the above steps as directed.

**ADDITION OF ANTI-HUMAN IgG PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION**

g) Using an 8 or 12-channel pipette (or transplating device) dispense 100 µl ready-to-use conjugate into each assay well. Discard pipette tips.

**Please Note:** Conjugate must be warmed to room temperature before use.

h) Incubate for 60 minutes at room temperature.

i) Wash as in steps e and f above.

j) Using as 8 or 12-channel pipette (or transplating device) dispense 100 µl Substrate Solution into each test well. Discard pipette tips.

k) Incubate at room temperature for 20 minutes.

l) Using an 8 or 12 channel pipette (or transplating device) add 100 µl ready-to-use Stop Solution to each test well.

m) Allow bubbles to dissipate before reading plate.

**MANUAL PROCESSING OF DATA**
a) Read the plate using an ELISA plate reader set at 405 with a 492 nm differential filter. Be sure to blank the reader as directed.

b) Calculate the average Positive Control Serum absorbance (Optical Density [O.D.]) using the absorbance values of wells A3 and A4. Calculate the average Normal Control Serum absorbance using values obtained from wells A1 and A2. Record both averages.

c) Subtract the average Normal Control Absorbance from the average Positive Control Absorbance. The difference is the Corrected Positive Control Absorbance.

d) Calculate a Sample to positive (Sp) ratio by subtracting the average Normal Control Absorbance from each Sample Absorbance. The difference is divided by the Corrected Positive Control Absorbance. Use the following equation format:

\[(\text{SAMPLE ABSORBANCE}) - (\text{AVERAGE NORMAL CONTROL ABSORBANCE}) \div \text{CORRECTED POSITIVE CONTROL ABSORBANCE}\]

Examples

1. Example Positive Control Absorbance (Wells A3 and A4): 0.456, 0.450
   Average = (0.456 + 0.450) / 2 = 0.453

2. Example Normal Control Absorbance (Wells A1 and A2): 0.060, 0.066
   Average = (0.060 + 0.066) / 2 = 0.063

3. Corrected Positive Control Absorbance: (0.453) - (0.063) = 0.390

4. Example Sp value calculation: Absorbance of sample = 0.585
   \[(0.585) - (0.063) \div 0.390 = 1.338\]

RESULTS

Assay Control Values

Valid VACCINIA ELISA results are obtained when the average optical density (O.D.) value of the Normal Control Serum is less than 0.150 and the Corrected Positive Control value range is between an OD of 0.3 and 0.6. If either of these values is out of range, the VACCINIA test results should be considered invalid and the samples should be retested.