ELISA TEST PROCEDURE

PREPARING THE TEST PLATE

a) Remove an anti-p27 antibody coated plate from the protective bag and label appropriately.
b) Directly add 100 µl Negative Control to wells A2, H10 and H12. Do not dilute. Discard pipette tips.
c) Directly add 100 µl Positive Control to wells A1, A3 and H11. Do not dilute. Discard pipette tips
d) Add approximately 100 µl unknown sample (or two drops of egg albumin) per well as per Figure 1. Start with well A4 and end with well H9 (moving left to right, row by row of wells). For example, wells 1 through 30 contain the samples from flock 1, wells 31-60 contain the samples from flock 2, etc.
e) Incubate plate for 30 minutes at room temperature. (21° to 24°C, 70° to 75°F)

WASH PROCEDURE

f) Tap out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.
g) Using an 8 or 12 channel pipette (or comparable automatic washing device), fill each well with approximately 300 µl Wash Solution. **Allow to soak in wells for 3 minutes;** 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 2 more times.**

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

ADDITION OF ANTI-p27 PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION

h) Using an 8 or 12 channel pipette (or transplating device) dispense 100 µl diluted conjugate (prepared as described above) into each assay well. Discard pipette tips.
i) Incubate for 30 minutes at room temperature. (21° to 24°C, 70° to 75°F)
j) **WASH** as in steps f and g above.
k) Using an 8 or 12 channel pipette (or transplating device) dispense 100 µl Substrate Solution into each test well. Discard pipette tips.
l) Incubate 15 minutes at room temperature. (21° to 24°C, 70° to 75°F)
m) Using an 8 or 12 channel pipette (or transplating device) add 100 µl diluted Stop Solution (prepared as described above) to each test well.
n) Allow bubbles to dissipate before reading plate.

MANUAL PROCESSING OF DATA

a) Read the plate using an ELISA plate reader set at 405 410 nm. Be sure to blank the reader as directed.
b) Calculate the average Positive Control absorbance (Optical Density [O.D.]) using the absorbance values of wells A1, A3 and H11. Calculate the average Negative Control absorbance values obtained from wells A2, H10 and H12. Record both averages.
c) Subtract the average negative absorbance from the average positive absorbance. The difference is the Corrected Positive Control.
d) Calculate a sample to positive (Sp) ratio by subtracting the average negative control absorbance from each sample absorbance. The difference is divided by the corrected positive control. Use the following equation format:

$$SP = \frac{\text{(SAMPLE ABSORBANCE) - (AVERAGE NEGATIVE CONTROL ABSORBANCE)}}{\text{CORRECTED POSITIVE CONTROL ABSORBANCE}}$$

RESULTS

Assay Control Values:

Valid ALV ELISA results are obtained when the average optical density (O.D.) value of the Negative Control is less than 0.250 and the Corrected Positive Control value range is between 0.150 and 1.200. If either of these values are out of range, the ALV test results should be considered invalid and the samples should be retested. Samples testing with an Sp value of less than 0.199 will receive a 0 titer value and are considered negative for p-27 antigen.

Under optimal conditions* the **suggested** O.D. value ranges of **0.06 to 0.20 for ALV Negative Control** and **0.50 to 1.00 for ALV Positive Control** should be strived for to ensure the most consistent laboratory test results. Please note that tests with O.D. values which do not fall within the suggested O.D. ranges above do not constitute an invalid test.

*Optimal conditions are at room temperature [70 to 75°F (21 to 24°C)]. Higher room temperatures may result in slightly higher O.D. values.

Interpretation of Results

Sp values reported by this system represent comparisons of the unknown antigen level of the sample to the positive control antigen. Therefore, it is important to first determine that the ALV ELISA positive and negative control values obtained are valid as detailed above in the "Assay Control Values" section of this pamphlet before ALV ELISA results are interpreted.

A "0" ALV ELISA value represents a chicken serum sample that contains an extremely low to insignificant p-27 antigen level compared to the ALV ELISA kit positive and negative controls.

An ALV ELISA value above "0" indicates only that a chicken serum sample contains a significant and ELISA-detectable p-27 level compared to the ALV ELISA kit positive and negative controls. However, these values do **not** imply or ensure "protection" nor provide serologic differentiation between an ALV vaccine response or an ALV field infection.

BIBLIOGRAPHY

1. Cottral, G.E., B.R. Burmester, and N.F. Waters. Egg Transmission of Avian Lymphomatosis. Poultry Sci. 33: 1174-84. 1954.
2. Fadly, A.M. Leukosis and Sarcomas. In: *Isolation and Identification of Avian Pathogens.* 3rd Edition. H.G. Purchase, L.H. Arp, C.H. Domermuth and J.E. Pearson eds. Kendall/Hunt Publishing Co., Dubuque, IA. Pp. 135-142. 1990.
3. Purchase, H.G. and L.N. Payne. Leukosis/sarcoma group. In: *Diseases of Poultry.* 8th Edition. M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid, and H.W. Yoder, Jr. Eds. Iowa State University Press, Ames, IA.

Please contact Synbiotics Technical Service at (800) 228-4305 or (858) 451-3771 with questions and comments.

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AVIAN LEUKOSIS VIRUS ANTIGEN TEST KIT

ITEM NO. 96-6555

SYNBIOTICS® CORPORATION

ProFLOK® PLUS

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U.S. VET LIC NO. 312

AVIAN LEUKOSIS VIRUS ANTIGEN TEST KIT

GENERAL INFORMATION AND INTENDED USES

Lymphoid leukosis, caused by Avian Leukosis Virus (ALV) is an insidious but economically important disease of chickens[2]. ALV infection may be associated with lymphoid leukosis tumors, decreased egg production and increased nonspecific mortality[2]. ALV is transmitted vertically from hen to chick through the egg and horizontally from bird to bird by direct or indirect contact[1,3].

The ProFLOK® Avian Leukosis Virus (ALV) antigen test kit offers a rapid method for the detection of ALV p27 antigen in chicken serum and egg albumin.

The assay is designed to measure antigen bound to anti-p27 antibody coated microtiter plates. The principle of the test is as follows: Samples collected from chickens infected with ALV contain specific ALV antigens, including p27. Serum or egg albumin samples are added to an anti-p27 antibody-coated plate. Specific antigen in the sample forms an antibody-antigen complex with the anti-p27 antibody bound to the plate. After washing the plate, an affinity purified rabbit anti-p27 peroxidase conjugate is added to each well. The antibody-antigen complex remaining from the previous step reacts with the conjugate. After a brief incubation period, the unreacted conjugate is removed by a second wash step. Substrate, which contains a chromagen (ABTS), is added to each well. Chromagen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. The relative intensity of color developed in 15 minutes (compared to controls) is directly proportional to the quantity of p27 antigen in the sample. After the substrate has incubated, Stop Solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at 405-410 nm.

REAGENTS REQUIRED TO PERFORM 90 TESTS

a) 1 p27 antibody coated plate
b) 300 µl ALV Positive Control
c) 300 µl Negative Control
d) 200 µl Rabbit anti-p27 Peroxidase Conjugate Solution
e) 10 ml Dilution Buffer
f) 10 ml ABTS-Hydrogen Peroxide Substrate Solution
g) 2.5 ml 5X Stop Solution, 5% SDS (dilute [1:5] with laboratory grade water)
h) 20 ml 20X Wash Solution (dilute [1:20] with laboratory grade water)

NOTE: Store all reagents provided in the kit at 2 to 7°C. Reagents should not be frozen.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

a) High precision pipette (i.e. 1-200 microliter pipette)
b) 0.2 ml, 1.0 ml and 5.0 ml pipettes
c) 8 or 12 channel pipette (or transplating device) and pipette tips
d) 2 graduated cylinders (50 ml)
e) Laboratory grade (Distilled or R.O.) water
f) 96 well plate reading spectrophotometer with 405-410 nm filter
g) Plate washing apparatus
h) Waste container with bleach or other oxidizing agent

WARNINGS TO THE USERS OF REAGENTS AND COATED PLATES

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 kit reagents should be properly decontaminated with bleach or other strong oxidizing agent before disposal.
d) Take special care not to contaminate any of the test reagents with serum or bacterial agents.
e) Humidity indicators are supplied with each plate. If any of the indicators exhibit a pink color, the plate may be compromised in some way; decontaminate (i.e. wash the plate with bleach solution) and dispose of the plate.
f) The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
g) Do not use this kit after the expiration date.
h) **NEVER PIPETTE BY MOUTH.**

ALLOW ALL REAGENTS TO COME TO ROOM TEMPERATURE (21° to 24°C, 70° to 75°F) BEFORE STARTING!

SAMPLE COLLECTION

For routine serologic flock monitoring, it is suggested that at least **30 or more sera or egg albumin samples per flock** be randomly collected at standard time intervals (i.e. every four weeks). 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

Serum or egg albumin samples may be added directly to the antibody coated plate without dilution. Frozen samples should be completely thawed and thoroughly mixed. Set up samples and controls as shown in Figure 1.

Preparation of Controls

An ALV Positive Control and a Negative Control have been provided with this kit in ready-to-use form. Allow the ALV Positive and Negative Control samples to equilibrate to room temperature before use.

NOTE: NO DILUTION OF THE ALV POSITIVE AND NEGATIVE CONTROLS IS NEEDED.

Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	-	+	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18	19	20	21
C	22	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44	45
E	46	47	48	49	50	51	52	53	54	55	56	57
F	58	59	60	61	62	63	64	65	66	67	68	69
G	70	71	72	73	74	75	76	77	78	79	80	81
H	82	83	84	85	86	87	88	89	90	-	+	-

Preparation of Conjugate Solution

The horseradish peroxidase conjugated Rabbit anti-p27 antibody is supplied in HRP Stabilizer. Dilute 200 µl stock conjugate in 10 ml Dilution Buffer (1:50 dilution). **Mix well.** This 10 ml preparation will supply sufficient conjugate for one 96 well ELISA plate.

Preparation of 1X Wash Solution

Dilute 20 ml concentrated Wash Solution in 380 ml laboratory grade (distilled or R.O.) water (1:20). **Mix well.** Approximately 400 ml Wash Solution is needed for each 96 well ELISA plate.

Preparation of the Substrate Solution

The Substrate Solution is ready to use. Each plate will require approximately 10 ml substrate solution. For example, 10 plates requires 100 ml substrate. **For best results, the substrate solution must be equilibrated to room temperature (21° to 24°C, 70° to 75°F) before use.**

Preparation of 1X Stop Solution

Dilute 2.5 ml concentrated Stop Solution in 10 ml laboratory grade (distilled or R.O.) water (1:5). **Mix well.** Approximately 12.5 ml Stop Solution is needed for each 96 well ELISA plate.

NOTE: Storage of 5X Stop Solution at refrigerated temperatures may cause the formation of a white solid. This does not affect product performance. Warm at room temperature (21° to 24°C, 70° to 75°F) or 37°C to dissolve before use.