

The Synbiotics ProFLOK® *Pasteurella multocida* (PM) antibody ELISA kit is a rapid and specific USDA-licensed serologic test for the detection of PM antibody in chicken serum samples.

PM is the causative agent of fowl cholera, a contagious septicemic disease of domestic poultry and wild birds that is often associated with high morbidity and mortality¹. The PM ELISA kit offers the poultry industry a reliable serologic test to monitor PM vaccination programs and an important screening tool for presumptive detection of PM infected flocks. The Synbiotics PM ELISA kit offers the following benefits and features:

- Reliable, consistent results
- USDA-licensed
- High-volume, easy-to-use format
- Long shelf life. All Synbiotics kits have an 18-month shelf life.
- Powerful database management software system
- Excellent field-oriented technical support

1. Specificity

The results shown in Table 1 below indicate that the Synbiotics PM ELISA kit demonstrates excellent specificity to PM antibody samples, but does not react significantly to other infectious agent antibodies. The Synbiotics PM ELISA kit, like all Synbiotics ELISA kits, is highly specific to provide valid, reproducible test results.

Table 1. Specificity.
Average sample-to-positive (SP) ratio values for an antigen specificity panel assayed with the SBIO PM ELISA test.

Antigen	Average SP ^{A,B} Ratio Values	SBIO PM ELISA SP Range ^C
Avian Pox virus (APV)	0.000	-
Avian reovirus (ARV)	0.000	-
Reticuloendotheliosis virus (REV)	0.000	-
Infectious bursal disease (IBD) virus	0.000	-
Infectious laryngotracheitis virus (ILT)	0.000	-
<i>Mycoplasma gallisepticum</i> (Mg)	0.000	-
<i>Mycoplasma synoviae</i> (Ms)	0.000	-
Newcastle disease virus (NDV)	0.000	-
Infectious bronchitis virus (IBV)	0.000	-
Avian encephalomyelitis (AE) virus	0.000	-
PM Type 3	1.220	+
PM Type 4	3.750	+
PM Type 5	3.210	+

^AValues are the arithmetic mean of 15 replicate samples (3 replicates / 5 ELISA plates)

^BSample SP Ratio = $\frac{\text{Optical Density (OD) sample} - \text{Avg. OD Normal Control}}{\text{Avg. OD Positive Control} - \text{Avg. OD Normal Control}}$

^CSBIO PM ELISA SP threshold ranges: Negative (-) = ≤ 0.199
Positive (+) = > 0.199

2. Sensitivity

The data shown in Table 2 demonstrate the sensitivity of the Synbiotics PM ELISA kit compared to the conventional PM AGP test. Additionally, the Synbiotics PM kit provides PM ELISA results within 3 hours compared to the 24 hours or more needed for the AGP test.

Table 2. Sensitivity.
Comparison of SBIO PM ELISA sample-to-positive (SP) ratio values, and titer values to the conventional PM agar gel precipitin (AGP) test for a PM sensitivity serum panel generated by serially diluting a high-titered PM serum in chicken normal control serum.

Dilution Ratio	SBIO PM SP Values ^{1,2}	SBIO PM ELISA Titer Values ³	AGP Results ⁴
1:1	4.168	12723	+
1:2	2.944	7648	+
1:4	1.900	4028	+
1:8	1.150	1931	+
1:16	0.638	815	-
1:32	0.346	333	-
1:64	0.170	118	-
R ² = 0.988			

¹Values are the arithmetic mean of 6 replicates.

²SBIO PM ELISA SP threshold ranges: Negative (-) = ≤ 0.199
Positive (+) = > 0.199

³Log₁₀ titer = $(1.464 \times \text{Log}_{10} \text{SP}) + 3.197$

⁴PM Agar gel precipitin (AGP) test results, based on - = weak to + = strong reaction scoring system. From a commercial laboratory.

3. Reproducibility

The data presented in Table 3 indicate that the Synbiotics PM ELISA kit demonstrates excellent reproducibility. All Synbiotics ELISA kits are optimized to provide valid, reproducible results test after test, day after day.

Table 3. Intra-plate and inter-plate reproducibility: Average sample-to-positive (SP) values and percent coefficient of variation (CV%) for high, medium, and low-titered PM sera assayed 30 times on each of five SBIO PM ELISA test plates.

SBIO PM ELISA Plate	High-Titered PM Serum		Medium-Titered PM Serum		Low-Titered PM Serum	
	SP ^A	CV(%)	SP ^A	CV(%)	SP ^A	CV(%)
1	1.985	6.21	0.921	4.23	0.389	5.90
2	2.034	5.92	0.894	4.67	0.371	6.24
3	1.952	6.53	0.921	7.07	0.386	5.95
4	1.920	5.98	0.845	5.76	0.359	6.37
5	2.021	6.23	0.913	6.08	0.377	4.96
Avg. Mean Values ^B	1.98	6.17	0.90	5.56	0.38	5.88

^AValues are the arithmetic mean of 30 replicates per PM ELISA kit plate.

^BValues are the arithmetic mean of 150 replicates per 5 PM ELISA kit plates.

Suggested Uses

1. PM Vaccination Evaluation:
 - Randomly collect and assay 30 or more serum samples per flock immediately prior to PM vaccine administration (pre-vaccination) and 14 to 18 days post vaccination.
2. Routine PM Flock Profiling:
 - Collect and assay 30 or more serum samples per flock, particularly breeder hen flocks, on a routine basis (i.e. every four to six weeks).

Interpreting Results

Evaluate Synbiotics PM ELISA results for the following:

1. Uniformity
 - Measured by the coefficient of variation value (CV%).
 - the lower the CV% value for a flock tested, the better the titer value uniformity.
 - Strive for the best 1-3 day old, pre-vaccination, and post-vaccination PM titer value uniformity (i.e. CV of less than 45%).

2. Titer Values

SP Threshold: Each Synbiotics ELISA kit has a sample-to-positive (SP) value threshold that clearly separates positive samples from negative samples. A negative sample is one that is not significantly different from the kit normal control serum. The SP threshold for the PM ELISA is as follows:

SP Range	Titer Range
0.199 or less	0
0.200 and greater	148 and greater

Vaccination Evaluation: Compare pre- and post-vaccination ELISA average mean titers, geometric mean titers and %CV values. A flock geometric mean titer following vaccination of 2500 is considered to be "protective". Note: Postvaccinal PM ELISA values depend on a variety of factors such as PM vaccine strain, route of administration, age of bird, etc. The goal of any vaccination program should be to achieve as uniform postvaccinal titer values for each vaccinated flock (i.e. %CV 45 or less) as possible.

Flock Profiling: Review ELISA flock profiles and correlate PM ELISA titer levels to vaccination program, flock economic performance data (body weight gain, feed conversion, mortality, etc.) and presence or absence of field infection.

Reference

1. Rhoades, K.R. and R.B. Rinler. Pasteurellosis. In: Diseases of Poultry, 9th ed. American Association of Avian Pathologists. Iowa State University Press, Ames, Iowa. pp. 145-171. 1991.