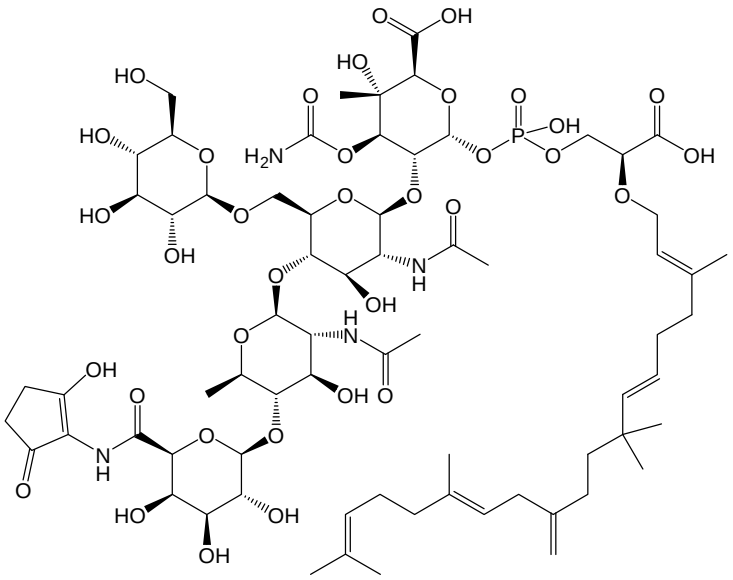
23 Flavophospholipol

(Flavomycin, Bambermycin)



Flavomycin

$C_{69}H_{107}N_4O_{35}P$ MW: 1584 CAS No.: 11015-37-5

[Summary of flavophospholipol]

Flavophospholipol (FV) is a phosphorus-containing polysaccharide antibiotic obtained by the incubation of *Streptomyces bambergiensis*, *Streptomyces ghanaensis*, *Streptomyces geysiriensis* and *Streptomyces ederensis*. It is a complex of several components having a chemical formula of $C_{65\text{ to }75}H_{124\text{ to }135}N_{6\text{ to }7}O_{40\text{ to }42}P$. When hydrolyzed, RV is devided into a lipid moiety (moenocinol, i.e, C_{25} alcohol with five primary double bonds), glucide moiety (D-glucose, D-glucosamine, D-quinovosamine and neutral glucide), and phosphorus moiety (phosphoric diester).

For physicochemical properties, FV technical occurs as brown to dark brown powder with a characteristic odor. It barely dissolves in methanol but insoluble in most of the other solvents. It is unstable in an acidic or basic solution and stable at pH 7 to 9.

FV has a strong antibacterial effect on Gram-positive bacteria and a growth promoting effect on chickens (including broilers) and pigs.

«Standards and specifications in the Act on Safety Assurance and Quality Improvement of Feeds»

FV is a feed-grade antibiotic that was designated as a feed additive as of July 24, 1976. The specifications for feeds containing this ingredient are specified in Appended Table No.1, 1-(1)-C of the Standards and Specifications in the Act on Safety Assurance and Quality Improvement of Feeds.

(in g(potency)/t)

Feed of interest	For chickens (except for broilers)	For broilers	For pigs	
	Starting chicks Growing chicks	Starting period broilers Finishing period broilers	Suckling piglets	Piglets
Added amount	1~5	1~5	2~10	2.5~5

[Methods listed in the Feed Analysis Standards]

1 Quantitative test method - Plate method (premix)

[Feed Analysis Standards, Chapter 9, Section 2, 23.1.1]

A. Reagent preparation

1) Buffer solution: Buffer No.7

2) Flavophospholipol standard solution. Weigh accurately not less than 40 mg of flavophospholipol working standard [1], accurately add a mixture of methanol and water (1:1) and dissolve to prepare a flavophospholipol standard stock solution with a concentration of 1 mg (potency)/mL[2].

At the time of use, accurately dilute a quantity of standard stock solution with Buffer No.7 to prepare high- and low-concentration standard solutions with concentrations of 2 and 0.5 μg (potency)/mL, respectively[3].

3) Clture medium: Medium F-12

At the time of use, add 1 mL each of methylene blue reagent and boric acid solution (4 w/v%) per 100 mL of culture medium[4].

4) Spore suspension and amount of addition. Use *Bacillus cereus* ATCC 19637 as the test organism. Add about 0.2 mL of the spore suspension with a concentration of 1×10^6 spores/mL per 100 mL of the culture medium.

5) Agar plate. Proceed by the agar well method.

B. Preparation of sample solution

1) When the analysis sample does not contain SL or MN

Weigh accurately 3 to 5 g of the analysis sample, place in a 200-mL stoppered Erlenmeyer flask, add 50 mL of acetone, stir for 20 minutes, add 50 mL of water, extract with stirring for 20 minutes, and filter the extract through filter paper (No.5A).

Accurately dilute a quantity of the filtrate with Buffer No.7 to prepare high- and low-concentration sample solutions with concentrations of 2 and 0.5 μg (potency)/mL, respectively[5].

2) When the analysis sample contains SL or MN[6]

Weigh accurately 3 to 5 g of the analysis sample, place in a 200-mL stoppered Erlenmeyer flask, add 50 mL of acetone, stir for 20 minutes, add 50 mL of water, extract with stirring for 20 minutes, and filter the extract through filter paper (No.5A).

Transfer accurately 25 mL of the filtrate to a 50-mL beaker, adjust the pH to 1.0 or lower, allow to stand for 1 hour, and again adjust the pH to 6.9 to 7.1 with ammonia solution. Transfer the whole amount

of this liquid with Buffer No.7 to a 50-mL volumetric flask, add Buffer No.7 up to the marked line, and filter through filter paper (No.5A).

Accurately dilute a quantity of filtrate with Buffer No.7 to prepare high- and low-concentration sample solutions with concentrations of 2 and 0.5 μg (potency)/mL, respectively[5].

C. Quantification[7]

Proceed by the 2-2 dose method[8], except that each agar plate shall be incubated at 27 to 29°C for 16 to 24 hours.

«Summary of analysis method»

This method is intended to determine the amount of FV in a premix by microbiological assay using a sample solution prepared by extracting with acetone and water and diluting with Buffer No.7. To remove the effect of SL or MN, premixes containing these ingredients shall be treated with hydrochloric acid.

The flow sheet of this method is shown in Figure 9.2.23-1.

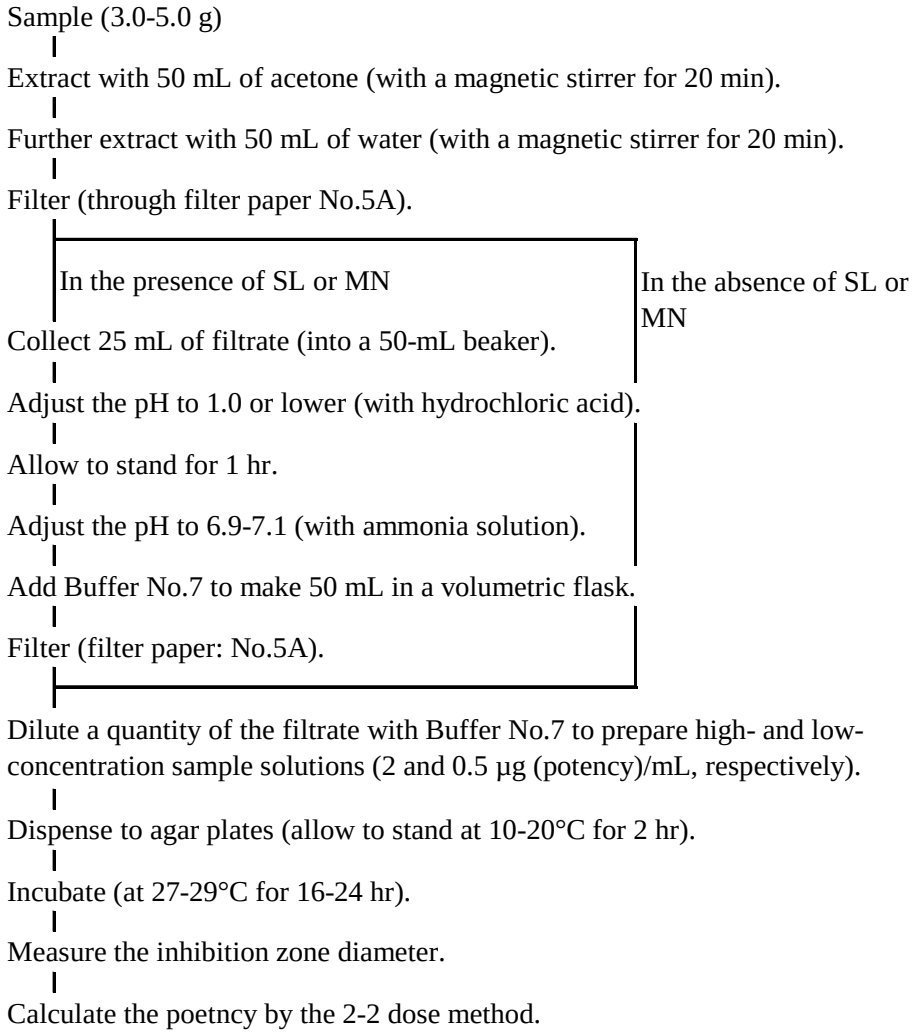


Figure 9.2.23-1 Quantitative test method for flavophospholipol (premix)

References: Noriyuki Koyama: Research Report of Animal Feed, 6, 163 (1980)

History in the Feed Analysis Standards [3] New

«Validation of analysis method»

・Spike recovery and repeatability

Sample type	Spike concentration (g(potency)/kg)	Repeat	Spike recovery (%)	Repeatability RSD(% or less)
Vitamin premix	0.5~2	3	99.4~101.2	2.2
Vitamin/mineral premix	0.5~2	3	99.0~100.2	1.8

«Notes and precautions»

[1] For the definition etc. of flavophospholipol working standard, refer to «Notes and precautions» [9] in Section 1, 1 of this Chapter.

[2] For the method of preparation for the standard stock solution, refer to «Notes and precautions» [10] in Section 1, 1 of this Chapter.

Method of preparation: Example (when the weighed amount is 50 mg)

When the labeled potency working standard is 987 μg (potency)/mg, 50 mg of the working standard contains 49,350 μg (potency) (i.e., 50 mg × 987 μg (potency)/mg). To prepare a standard stock solution with a concentration of 1,000 μg (potency)/mL, the require amount of solvent is thus calculated to be 49.35 mL (i.e., 49,350 μg (potency) / 1,000 μg (potency)/mL). Therefore, completely transfer 50 mg working standard to an Erlenmeyer flask containing 49.35 mL of a mixture of methanol and water (1:1) and dissolveto prepare the standard stock solution with a concentration of 1,000 μg (potency)/mL.

[3] For the method of preparation for the standard solution, refer to «Notes and precautions» [8] in Section 1, 1 of this Chapter.

An example method of preparation for flavophospholipol standard solution is shown in Table 9.2.23-1.

[4] Prepare a solution of 0.1 g of methylene blue in 100 mL of methanol and a solution of 4 g of boric acid in 100 mL of sterile water, and add 1 mL each of these solutions per 100 mL of the culture medium previously melted under sterile conditions.

This procedure makes the inhibition zone blue and the outside the zone white, facilitating the measurement of the inhibition zone.

[5] For the method of preparation for the sample solution, refer to «Notes and precautions» [8] in Section 1, 1 of this Chapter.

An example method of preparation is shown in Table 9.2.23-1.

Table 9.2.23-1 Method of preparation for flavophospholipol standard solution and sample solution

1) Method of preparation for flavophospholipol standard solution (premix, example)

Test tube No.	1	2	3	4
Amount (mL) of standard solution	②	2	4	5
Amount (mL) of Buffer No.7	18	18	16	15
Concentration (μg(potency)/mL)	100	10	2	0.5

Note: "② mL" means "2 mL of standard stock solution (1 mg(potency)/mL)".

2) Method of preparation for sample solution (premix not containing SL or MN, example)

When the analysis sample is collected in an amount equivalent to 10,000 μg (potency) of FV, the concentration of FV in the filtrate is calculated to be 100 μg (potency)/mL.

Test tube No.	1	2	3
Amount (mL) of sample solution	②	4	5
Amount (mL) of Buffer No.7	18	16	15
Concentration (μg(potency)/mL)	10	2	0.5

Note: "②mL" means "2 mL of the filtrate (100 μg(potency)/mL)".

[6] Under the considions of this assay, the test organism *Bacillus cereus* ATCC 19637 becomes sensitive to SL or MN when their content in the sample solution is not less than 10 μg (potency)/mL. To remove this effect, treat the sample solution with hydrochloric acid if the analysis sample contains SL or MN in combination with flavophospholipol. Refer to «Notes and precautions» [8] in 1. Zinc bacitracin or manganese bacitracin 1.1 of this Section.

[7] An example standard response line for FV is shown in Figure 9.2.23-2.

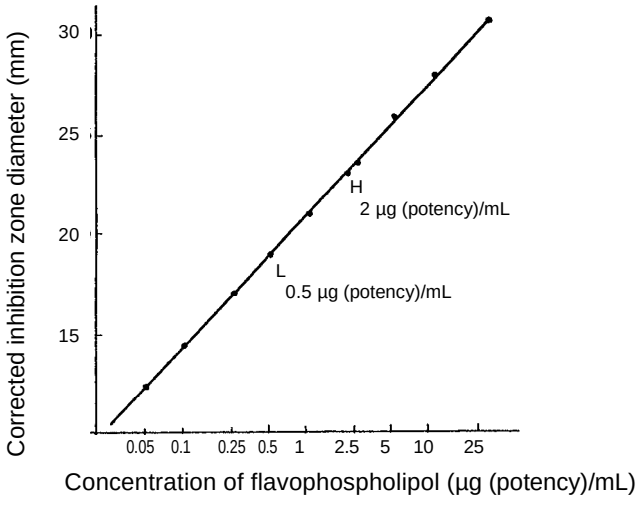


Figure 9.2.23-2 Standard response line for flavophospholipol (premix , example)
(*Bacillus cereus* ATCC 19637, Medium F-12, Agar well method)

[8] Refer to «Notes and precautions» [53] to [60] in Section 1, 1 of this Chapter.