Technical Information

Antibiotic Assay Medium L- AOAC

Product Code: DM 1991

Application: - Antibiotic Assay Medium L is recommended by AOAC for microbiological assay of Monensin using *Bacillus subtilis* ATCC 6633 as test organism.

Composition**

Ingredients	Gms / Litre
Dipotassium hydrogen phosphate	0.690
Monopotassium phosphate	0.450
Yeast extract	2.500
Dextrose, anhydrous	10.000
Agar	15.000
Final pH (at 25°C)	6.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Antibiotic Assay Medium L is formulated in accordance with AOAC [(1)] for the microbiological assay of Monensin in feeds, using *Bacillus subtilis* (ATCC 6633) as the test organism.

Use single inoculated agar layer. Optimum concentration of suspension of *Bacillus subtilis,* is determined before assay by preparing trial plates. To obtain appropriate inhibition zones (17.5 ± 2.5 mm with 0.5µg/ml) usually 0.5 ml suspension is used per 100 ml of seed agar For actual assay appropriate amount of suspension is added to sterile, molten medium, mix and pour 6 ml into sterile Petri plate. Cover and refrigerate for about 1 hour before use.

For the standard graph or response lines prepare dilution using 50% methanol to obtain 0.25, 0.5, 1.0 and 2.0 µg monensin/ml. Reference concentration is 0.5 µg/ml. To obtain standard curve 10 seeded agar plates are used placed with cylinders. Different standard concentrations are filled in it. Incubate at 16 18 hours at 35-37°C and measure diameters of zones of inhibition. Weigh 20gram finished feed and 5 gram premix and add it to chromatographic column. Elute with 9:1 methanol water. 200 ml elute is again diluted with 50% methanol to 0.5µg monensin /ml. This is called assay solution. Use 5 plates for each assay solution. Fill the alternate cylinders with reference concentration and assay solution after incubation at 35-37°C for 16-18 hours, measure diameters of zones of inhibition to nearest 0.1 mm. Average 10 reading of reference concentration and 10 reading of reference concentration and 10 readings of assay solution.

If assay solution gives larger average than reference concentration adds difference between them to reference point on standard curve. If assay solution gives smaller value than reference concentration, substract difference between them from reference point. Using corrected value of assay solution determine amount of antibiotic.

Methodology

Suspend 28.64 grams of powder media in 1000 ml. distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 2.86% w/v aqueous solution at 25°C. pH : 6.0±0.2

Ph range 5.80-6.20

Cultural Response/ characteristices
DM 1991: Cultural characteristics observed after an incubation at 35 - 37°C f or 16 - 18 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Anibiotic assayed
Bacillus subtilis ATCC 6633	50-100	luxuriant	>=70%	Inhibition zones with Monensin

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Williams. (Ed.), 2005, Official Methods of Analysis of AOAC International, 19th ed., AOAC, International, Washington D. C.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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