Antibiotic Assay Medium L- AOAC

M991

Intended Use:

Recommended for microbiological assay of Monensin using *Bacillus subtilis* as test organism.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 28.64 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And 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. Usually 0.5 ml suspension is used per 100 ml of seed agar, to obtain appropriate inhibition zones (17.5± 2.5 mm with 0.5µg/ml). For actual assay add appropriate amount of suspension 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 add 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.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling:

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

Freshly prepared medium plates must be used ps u nb sftvmu o fsspofpvt sftvmut / tf pg ui t nfuipe t b sp s buf pom ifo uftu tbn mft bsf dmfbs/

Quality Control

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
5.80-6.20

Cultural Response
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

Reference

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

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