

Riboflavin Assay Medium

Intended Use

Riboflavin Assay Medium is used for determining riboflavin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Riboflavin Assay Medium is a modification of the medium described by Snell and Strong.¹ It is recommended for use in the microbiological assay of riboflavin following the method-

ology outlined in the *Official Methods of Analysis of AOAC International*² using *Lactobacillus rhamnosus* ATCC™ 7469 as the test organism.

Principles of the Procedure

Riboflavin Assay Medium is free from riboflavin but contains all other nutrients and vitamins essential for the growth of *Lactobacillus rhamnosus* ATCC 7469. The addition of riboflavin in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Riboflavin Assay Medium

Approximate Formula* Per Liter

Dextrose	20.0	g
Sodium Acetate	15.0	g
Vitamin Assay Casamino Acids	10.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
L-Asparagine	0.6	g
DL-Tryptophan	0.2	g
L-Cystine	0.2	g
Magnesium Sulfate USP	0.4	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Xanthine	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate (monohydrate)	20.0	mg
Sodium Chloride USP	20.0	mg
Pyridoxine Hydrochloride	4.0	mg
Pyridoxal Hydrochloride	4.0	mg
p-Aminobenzoic Acid	2.0	mg
Calcium Pantothenate	800.0	µg
Folic Acid	800.0	µg
Nicotinic Acid	800.0	µg
Thiamine Hydrochloride	400.0	µg
Biotin	1.0	µg

*Adjusted and/or supplemented as required to meet performance criteria.

User Quality Control

Identity Specifications

Difco™ Riboflavin Assay Medium

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 2.4% solution (single strength) and 4.8% (double strength), soluble in purified water upon boiling. Solution is light to medium amber, clear, may have a slight precipitate.

Prepared Appearance: Light amber, clear, may have a very slight precipitate.

Reaction of 2.4% Solution at 25°C: pH 6.8 ± 0.2

Cultural Response

Difco™ Riboflavin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus rhamnosus* ATCC™ 7469 when prepared in single strength and supplemented with riboflavin. The medium should produce a standard curve when tested using a riboflavin reference standard at 0.0 to 125.0 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware, free from detergents and other chemicals, must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilizing and cooling conditions uniform throughout assay.

Directions for Preparation from Dehydrated Product

1. Suspend 4.8 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the volume to 10 mL with purified water.
6. Autoclave at 121°C for 10 minutes.

Procedure

Follow applicable assay procedures.² Levels of riboflavin used in the determination of the standard curve should be prepared according to this reference or according to the following procedure.

Stock Cultures

Stock cultures of *L. rhamnosus* ATCC 7469 are prepared by stab inoculation into 10 mL of Lactobacilli Agar AOAC. After 24-48 hours incubation at 35-37°C, the stock cultures are kept in the refrigerator. Transfers are made at monthly intervals in triplicate.

Inoculum

Inoculum for assay is prepared by subculturing a stock culture of *L. rhamnosus* ATCC 7469 into 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. Following incubation for 16-24 hours at 35-37°C, the culture is centrifuged under aseptic conditions and the supernatant liquid decanted. After washing 3 times with 10 mL sterile 0.85% saline, the cells are resuspended in 10 mL sterile 0.85% saline. The cell suspension is then diluted with sterile 0.85% saline, to a turbidity of 35-40% transmittance when read on the spectrophotometer at 660 nm. One drop of this latter suspension is then used to inoculate each of the assay tubes.

Riboflavin Assay Medium may be used for both turbidimetric and titrimetric determinations. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C, whereas titrimetric determinations are best made after 72 hours incubation at 35-37°C. Using Riboflavin Assay Medium, the most effective assay range is between 0.025 and 0.15 µg riboflavin.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation, which influence the standard curve readings, cannot be duplicated exactly from assay to assay. The

standard curve is obtained by using Riboflavin USP Reference Standard or equivalent at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 µg riboflavin per assay tube (10 mL).

The concentration of riboflavin required for the preparation of the standard curve may be prepared by dissolving 0.1 g of Riboflavin USP Reference Standard or equivalent in 1,000 mL of purified water by heating, giving a stock solution of 100 µg per mL. Dilute the stock solution by adding 1 mL to 999 mL purified water. Use 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 mL of the diluted stock solution per tube. Prepare the stock solution fresh daily.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary by more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.
5. Maintain pH below 7.0 to prevent loss of riboflavin.

References

1. Snell and Strong. 1939. Ind. Eng. Chem. 11:346.
2. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Riboflavin Assay Medium

AOAC

Cat. No. 232510 Dehydrated – 100 g*

*Store at 2-8°C.