CM 20727 – FOLIC ACID MEDIUM, AOAC

INTENDED USE

For the microbiological assay of folic acid using Enterococcus hirae ATCC 8043.

PRODUCT SUMMARY AND EXPLANATION

Folicacidisrequiredforthegrowth of Enterococcus hirae. Hence growth of this organism will occur only if the sample beingassayedcontainsfolicacid.The exact folic acid concentration in the test sample can be determined by comparing thegrowthobtainedtothatofknown standard concentrations of folic acid (standard curve).
FolicAcidAssayMediumisprepared according to the formula described by Capps et al and is recommended by AOAC for thedeterminationoffolicacidcontent of the pharmaceutical products and other materials using Enterococcus hirae ATCC 8043asthetest organism.
Procedure:StockculturesofEnterococcus hirae ATCC 8043 are prepared by stab inoculation of Lactobacilli Agar AOAC. Followingincubationat35-37°Cfor 24 hours, the tubes are stored in the refrigerator. Transplants are made at monthly intervals.Inoculumforassayisprepared by sub culturing from a stock culture of Enterococcus hirae ATCC 8043 into a tubecontaining10mlofLactobacilli Broth AOAC. After 24 hours incubation at 35-37°C, the cells are centrifuged under asepticconditions,andthesupernatant liquid is decanted. The cells are resuspended in 10 ml of sterile 0.85% NaCl. The cellsuspensionisthendiluted1:100 with sterile 0.85% NaCl. One drop of this later suspension is used to inoculate each oftheassaytubes.Itisessentialthat a standard curve be set up for each separate assay since conditions of autoclaving, temperatureofincubation,etc.,which influence the standard curve readings cannot be duplicated exactly from time to time.Thestandardcurveisobtained by using folic acid at levels of 0, 2, 4, 6, 8 and 10 ng per assay tube (10 ml). Tubes arerefrigeratedfor15-30minutes to stop growth before reading. Turbidimetric readings should be read after 16-18 hoursincubationat35-37°CandAcidimetric after 72 hours at 35-37°C. To prepare stock solution of folic acid, 20 mg folic acid isused.
PreparationofFolicAcidConcentrations:
Dissolve20mgdriedfolicacidin100 ml distilled water containing 20 ml ethanol. Adjust the pH of the solution to 10.0 with0.1NNaOHtodissolvetheacid and then adjust pH to 7.0 with 0.05 N HCl. This solution contains 200 mcg folic acid perml.Dilute1mlofthissolutionwith 999 ml of distilled water to get 200 ng per ml and finally, dilute 1 ml of this solution with999mlofFolicAcidBufferAto get a standard solution containing 0.2 ng folic acid per ml. use 0.0, 0.5, 1.0, 2.0, 3.0, 4.0and5.0mlperassaytube.Extreme care should be taken to avoid contamination of media or glassware used for the assay.Detergentfreecleanglassware should be used. Even small amount of contamination by foreign material can be lead to erroneousresults.

COMPOSITION

Ingredients	Gms / Ltr
Vitamin free Casein acid hydrolysate	10.000
L-Asparagine	0.600
L-Tryptophan	0.200
L-Cystine hydrochloride	0.760
Dextrose (Glucose)	40.000
Adenine sulphate	0.010
Guanine hydrochloride	0.010
Uracil	0.010

Xanthine	0.020
p-Amino benzoic acid (PABA)	0.001
Pyridoxine hydrochloride	0.004
Thiamine hydrochloride	0.0004
Calcium pantothenate	0.0008
Nicotinic acid	0.0008
Biotin	0.00002
Riboflavin (Vitamin B2)	0.001
Glutathione	0.0052
Polysorbate 80	0.100
Sodium citrate	52.000
Dipotassium hydrogen phosphate	6.400
Magnesium sulphate	0.400
Manganese sulphate	0.020
Sodium chloride	0.020
Ferrous sulphate	0.020

PRINCIPLE

The medium consists of Vitamin Free Casein acid hydrolysate, Dextrose, L-cystine which provide the necessary organic carbon and nitrogen source. Magnesium and sulphates are a cofactor for many metabolic reactions. Sodium chloride in the medium maintains the osmotic balance. Folic Acid Assay Medium contains all the necessary nutrients for the growth of the test organism except folic acid. The medium contains nutrients like amino acids, carbohydrates, purine, pyrimidines, salts, and vitamins.

INSTRUCTION FOR USE

Dissolve 11.1grams in 100 ml distilled water.
Heat to boiling for 2-3 minutes. Agitate to dispense the slight precipitate evenly.
For assay, dispense 5 ml medium into each assay tube (containing increasing amounts of standard or unknown) and make up the total volume to 10 ml per tube with distilled water.
Sterilize by autoclaving at 15 psi pressure (121°C) for 5 minutes. Cool to 45-50°C. Satisfactory results are obtained with Folic acid at levels of 0, 2, 4, 6, 8, 10 nanograms per assay tube.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Light amber coloured, clear solution, which may have slight precipitate.
pH (at 25°C)	: 6.7 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Enterococcus hirae	8043	50-100	Good Gradual increase in growth with increasing standard Folic acid concentration 0,2,4,6,8,10 ng per assay tube is recorded as equivalent increase in absorbance at 620nm	35-37°C	18-24 Hours

PACKAGING:

Inpacksizeof100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

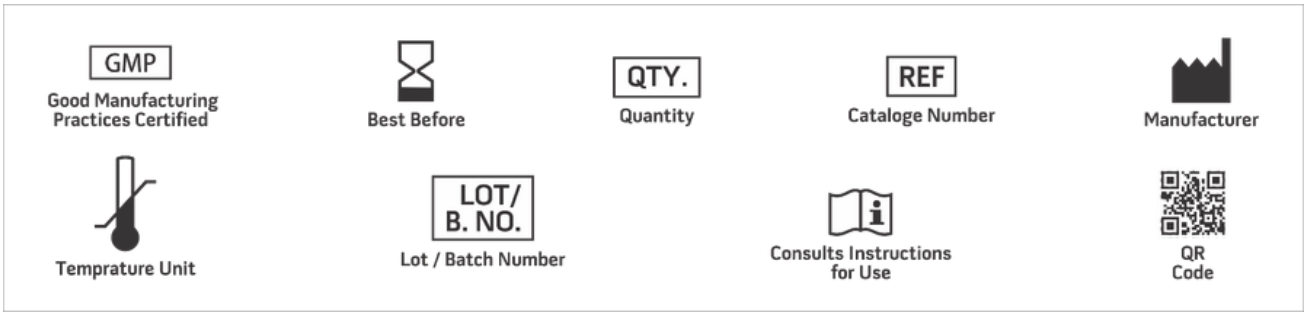
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

Afteruse,prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1.Capps,Hobbs and Fox, 1948, J. Bacteriol., 55:869.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Official Methods of Analysis of AOAC International, 2005, 19th Ed., Vol. II, Association of Analytical Chemists, Washington, D.C.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*For LabUse Only