TM 2086 – FOLIC ACID ASSAY MEDIUM

INTENDED USE

For microbiological assay of Folic Acid using *Enterococcus hirae* ATCC 8043 as the test organism.

PRODUCT SUMMARY AND EXPLANATION

Folic Acid Assay Medium is prepared according to the formula described by Capps et al and is recommended for the determination of folic acid content of the pharmaceutical products. Folic acid is required for the growth of *Enterococcus hirae*. Hence growth of this organism will occur only if the sample being assayed contains folic acid. The exact folic acid concentration in the test sample can be determined by comparing the growth obtained to that of known standard concentrations of folic acid (standard curve).

Procedure:

Stock cultures of *Enterococcus hirae* ATCC 8043 are prepared by stab inoculation of Micro Vitamin Test Culture Agar. Following incubation at 35-37°C for 24 hours, the tubes are stored in the refrigerator. Transplants are made at monthly intervals. Inoculum for assay is prepared by sub-culturing from a stock culture of *Enterococcus hirae* ATCC 8043 into a tube containing 10 ml of Micro Vitamin Test Inoculum Broth. After 24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions, and the supernatant liquid is decanted. The cells are resuspended in 10 ml of sterile 0.85%NaCl. The cell suspension is then diluted 1:100 with sterile 0.85% NaCl. One drop of this later suspension is used to inoculate each of the assay tubes. It is essential that a standard curve be set up for each separate assay since conditions of autoclaving, temperature of incubation, etc., which influence the standard curve readings cannot be duplicated exactly from time to time. The standard curve is obtained by using folic acid at levels of 0, 2, 4, 6, 8 and 10 ng per assay tube (10 ml). Tubes are refrigerated for 15-30 minutes to stop growth before reading. Turbidimetric readings should be read after 16-18 hours incubation at 35-37°C and acidimetric after 72 hours at 35-37°C. To prepare stock solution of folic acid, 20 mg folic acid is used.

Preparation of Folic Acid Concentrations:

Dissolve 20 mg dried folic acid in 100 ml distilled water containing 20 ml ethanol. Adjust the pH of the solution to 10.0 with 0.1 N NaOH to dissolve the acid and then adjust pH to 7.0 with 0.05 N HCl. This solution contains 200 mcg folic acid per ml. Dilute 1 ml of this solution with 999 ml of distilled water to get 200 ng per ml and finally, dilute 1 ml of this solution with 999 ml of Folic Acid Buffer A to get a standard solution containing 0.2 ng folic acid per ml. use 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml per assay tube. Extreme care should be taken to avoid contamination of media or glassware used for the assay. Detergent free clean glassware should be used. Even small amount of contamination by foreign material can be lead to erroneous results.

COMPOSITION

Ingredients	Gms / Ltr
Casein acid hydrolysate, vitamin free	12.000
Dextrose	40.000
Sodium citrate	20.000
L-Cystine	0.200
DL-Tryptophan	0.200
Adenine sulphate	0.020
Guanine hydrochloride	0.020
Uracil	0.020
Thiamine hydrochloride	0.002
Pyridoxine hydrochloride	0.004
Riboflavin (Vitamin B2)	0.002

Niacin	0.002
p-Amino benzoic acid (PABA)	0.0002
Biotin	0.0000008
Calcium pantothenate	0.0004
Dipotassium phosphate	1.000
Monopotassium phosphate	1.000
Magnesium sulphate	0.400
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.020

PRINCIPLE

The medium consists of Casein acid hydrolysate, vitamin free, Dextrose, L-cystine which provide the necessary organic carbon and nitrogen source. The medium contains citrate and phosphate as buffer salts. 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 75.0 grams in 1000 ml distilled water.
- Heat if necessary to dissolve the medium completely.
- Mix well to distribute the slight precipitate evenly.
- For assay, dispense 5 ml medium per assay tube (containing increasing amounts of standard or the unknown) and make total volume to 10 ml with distilled water.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 10 minutes. Cool immediately. Satisfactory results are obtained with Folic Acid at levels of 0,2,4,6,8 and 10mg per assay tube (10ml).

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Off-white to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light amber coloured, clear solution, which may contain a slight precipitate.
pH (at 25°C) : 6.8 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Enterococcus hirae	8043	50-100	Good growth is obtained. Gradual increase in growth with increasing conc. of standard folic acid 0, 2, 4, 6, 8, 10 ng per assay tube was recorded as equivalent increase in absorbance at 620 nm	35-37°C	16-18 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°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.

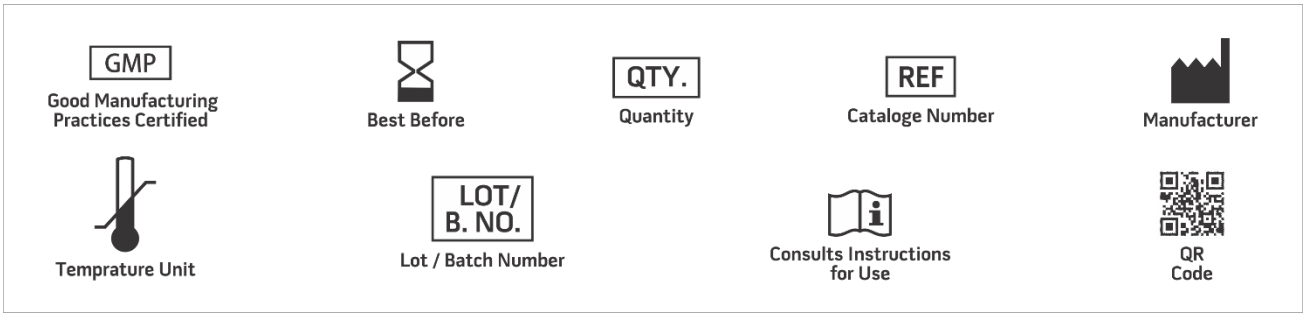
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

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

REFERENCES

1. Capps, Hobbs and Fox, 1948, J. Bact., 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 Lab Use Only**

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