CM 20724 – FOLIC ACID CASEI MEDIUM, MODIFIED

INTENDED USE

For the microbiological assay of folic acid in blood serum using Lactobacillus casei ATCC 7469 as the test organism.

PRODUCT SUMMARY AND EXPLANATION

Folic Acid Casei Medium is used for the microbiological assay of folic acid in blood serum using Lactobacillus casei ATCC 7469 as the test organism. This medium is based on the formulation of Flynn et al, modified by Baker et al and Waters and Mollin. The test organism used in vitamin assay generally requires three media, i.e. a culture maintenance medium, an inoculation medium and a test medium. The latter is usually a chemically defined medium that contains all the ingredients and nutrients essential for growth of the test organisms except the material under study. Similarly, Folic Acid Casei Medium contains all the essential nutrients for the growth of L. casei except folic acid. Therefore, additions of folic acid in specified increasing concentrations gives a similar increase in the growth response of L. casei.

Technique

Stock cultures of Lactobacillus casei ATCC 7469 are prepared by stab inoculation of Lactobacilli Agar AOAC. Following incubation at 35-37°C for 18-24 hours the tubes are stored in a refrigerator. Transfers are made at monthly intervals. Inoculum for assay is prepared by sub culturing from stock culture of Lactobacillus casei ATCC 7469 into a tube containing 10 ml of Micro Vitamin Test Inoculum Broth or Lactobacilli 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 then resuspended in 10 ml of sterile single strength Folic Acid Casei Medium, Modified resedimented as before and washed one more time. Finally, the washed cells are resuspended in 10 ml of sterile single strength Folic Acid Casei Medium, Modified and diluted 1:100 with the same medium. One drop of this suspension is used to inoculate each of the assay tubes. 0.85% NaCl can be used instead of the single strength basal medium to wash and dilute the inoculum. A standard curve for each assay should be prepared, since the conditions of sterilization, 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 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ng per assay tube (10 ml).

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 0.1N NaOH to dissolve the acid and then adjust pH to 7.0 with 0.05N HCl. This solution contains 200 mcg folic acid per ml. Dilute 1 ml of this solution with 999 ml of distilled water to get 200ng 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 ml per assay tube.

Preservation of Serum Specimen

Allow the blood specimen to clot so as to separate the serum. Separate the serum into a clean dry tube and centrifuge to remove any blood cells present. Take care to avoid haemolysis of erythrocytes. Dispense 5 ml of serum sample into clean dry test tubes and add 25 mg ascorbic acid to each tube. Keep the tubes frozen below -20°C till assay.

Preparation of Serum Specimens

Thaw the serum containing ascorbic acid. Add 5 ml of this sample to 45 ml of rehydrated Folic Acid Buffer A. Incubate this serum - buffer solution at 37°C for 90 minutes and then autoclave at 15 psi pressure (121°C) for 2.5 minutes. Remove the coagulated protein by centrifuging and transfer the supernatant to a clean, dry tube. This clear solution obtained is used as a sample in the folic acid assay.

Procedure for determination of Total Folic Acid concentration in specimens

Use 0.5, 1.0, 1.5 ml or other volumes of the prepared serum extracts as described earlier. Fill each tube with 5 ml Folic Acid Casei Medium, Modified and sufficient distilled water to give total volume of 10 ml per tube. Sterilize tubes at 15 lbs pressure (121°C) for 5 minutes. Cool the tubes and add one drop of inoculum to each assay tube. Turbidimetric reading should be made after 18-24 hours incubation at 35-37°C. Tubes are refrigerated for 15-30 minutes to stop growth before reading. The turbidometric readings are recorded at 620 nm. The amount of folic acid in the test samples can be determined by interpreting the results with the values obtained on the standard curve taking into consideration the dilution of sample. 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 erroroneus results.

COMPOSITION

Ingredients	Gms / Ltr
Charcoal Treated Tryptone	10.000
Dextrose (Glucose)	40.000
Sodium acetate	40.000
Dipotassium hydrogen phosphate	1.000
Potassium dihydrogen phosphate	1.000
DL-Tryptophan	0.200
L-Asparagine	0.600
L-Cysteine hydrochloride	0.500
Adenine sulphate	0.010
Guanine hydrochloride	0.010
Uracil	0.010
Xanthine	0.020
Polysorbate 80 (Tween 80)	0.100
Glutathione reduced	0.005
Magnesium sulphate	0.200
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.015
Riboflavin (Vitamin B_2)	0.001
p-Amino benzoic acid (PABA)	0.002
Pyridoxine hydrochloride	0.004
Thiamine hydrochloride	0.0004
Calcium pantothenate	0.0008
Nicotinic acid	0.0008
Biotin	0.00002

PRINCIPLE

The medium consists of Tryptone, Dextrose, L-cystine which provide the necessary organic carbon and nitrogen source. The medium contains phosphate as buffer salts. Magnesium and sulphates are a cofactor for many metabolic reactions.

Sodium chloride in the medium maintains the osmotic balance. The medium contains nutrients like amino acids, carbohydrates, purine, pyrimidines, salts, and vitamins.

INSTRUCTION FOR USE

Dissolve 9.372 grams in 100 ml distilled water containing 50 mg ascorbic acid.
Boil for 1-2 minutes to dissolve the medium completely.
Dispense 5 ml amount in test tubes. After adding standard and test solution adjust volume in test tube to 10 ml with purified / distilled water.
Sterilize by autoclaving at 15 psi pressure (121°C) for 5 minutes.
Standard curve is obtained using Folic acid at levels of 0, 0.1, 0.2, 0.4,0.6, 0.8 and 1ng 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 have a slight precipitate.
pH (at 25°C)	: 6.7 ± 0.2

INTERPRETATION

Culturalcharacteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Lactobacillus casei	7469	50-100	Good Gradual increase in growth with increasing concentration of standard folic acid 0, 0.1 ,0.2, 0.4, 0.6, 0.8 and 1ng per assay tube was recorded as equivalent increase in absorbance at 620 nm	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 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

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

REFERENCES

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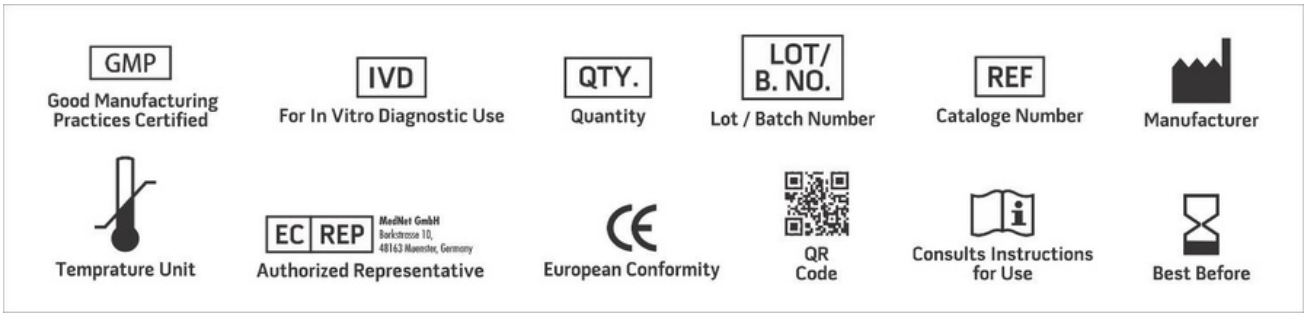
2. Flynn, Williams, ODell and Hogan, 1951, Anal. Chem., 23, 180.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. Waters and Mollin, 1961, J. Clin. Path., 14, 335.



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

*For LabUse Only