CM 20209 – *B12 ASSAY AGAR (using E.coli mutant Culture)*

INTENDED USE

For microbiological assay of Vitamin B12 by plate method using E.coli mutant as test organism.

PRODUCT SUMMARY AND EXPLANATION

B12AssayAgar is dehydrated medium devoid of Vitamin B12 but containing all the nutrients essential for the growth of E. coli mutant 113-3 Davis ATCC-11105. For the preparation of Standard, make sterile solutions of Vitamin B12 (Cyanocobalamine Reference Standard). For the determination of Vitamin B12 content of unknown materials the assay sample should be properly diluted and applied similarly as the dilutions of the standards. Inoculum for the assay is prepared by sub-culturing from a stock culture previously made by stab inoculation. Freshly subcultured cells incubated at 35°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for this assay.

COMPOSITION

Ingredients	Gms / Ltr
Dipotassium Phosphate	14.000
Monopotassium Phosphate	6.000
Ammonium Chloride	5.000
Glucose	5.000
DL- Asparagine	3.000
Ammonium Nitrate	2.000
Sodium Chloride	1.000
Magnesium Sulphate	0.200
L- Arginine Hydrochloride	0.200
Ammonium Sulphate	0.100
Calcium Chloride	0.001
Zinc Sulphate	0.00009
Ammonium Molybdate	0.00001
Borax	0.00001
Ferrous Sulphate	0.000054
Manganese Chloride	0.000046
Copper Sulphate	0.000025
Agar	15.000

PRINCIPLE

Themediumcontains glucose which acts as a source of energy. Incorporation of Vitamin B12 in specified increasing amounts gives a growth response that can be measured by the diameter of the zone of growth around the disc or cup containing Vitamin B12. Phosphates present provide ions to the medium. And other salts help in maintaining the osmotic balance. Agar acts as a solidifying agent.

INSTRUCTION FOR USE

Dissolve51.5gramsin1000 ml purified / distilled water.

Heat to boiling to dissolve the medium completely.
Mix well to distribute slight precipitate evenly.
Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
Generally satisfactory results are obtained with B12 at levels ranging from 0 to 300 ng per ml.

Caution: Over heating or over sterilization will give unsatisfactory results.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream coloured, homogenous, free flowing powder with a tendency to clump.
Appearance of prepared medium	: Beige to light amber coloured, slightly opalescent gel forms in petri plates.
pH (at 25°C)	: 7.2 ± 0.2

INTERPRETATION

Culturalcharacteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Escherichia coli 113-3D	11105	50-100	Good	40-50%	30ºC -35°C	18-24 Hours

PACKAGING:

Inpacksizeof100 gm bottles.

STORAGE

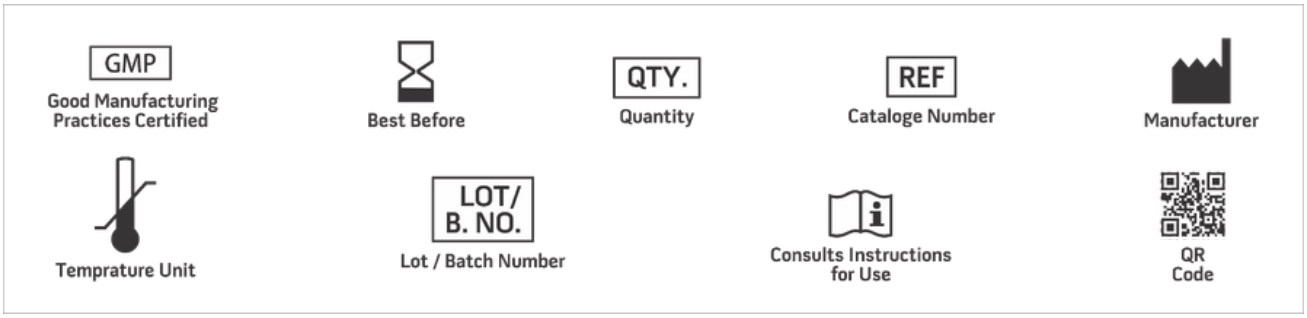
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided 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

1. USPharmacopeial Convention, Inc. 2001. The Unites States Pharmacopeia 25/NF20-2002. The US Pharmacopeia Convention, Inc; Rockville, Md.
2. Harrison, E., Lees, K.A and Wood, F. (1951) Analyst 76: 696.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd



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

*For LabUse Only