B12 Assay Agar (using E. coli Mutant Culture)

Intended Use
B12 Assay Agar (using E. coli mutant culture) is recommended for the microbiological assay of Vitamin B12 by the cup plate or disc plate method.

Summary
B12 Assay is carried out according to the procedures of the vitamin B12 Activity Assay as per the USP. *E. coli* mutant 113-3D is the test organism used in this assay.

Principle
B12 Assay Agar is a dehydrated medium devoid of Vitamin B12, containing all the other nutrients essential for the growth of *E. coli* mutant 113-3D ATCC 11105. Incorporation of Vitamin B12 in specified increasing concentrations gives a growth response that can be quantified by measuring the diameter of the zone of growth around the disc or cup containing Vitamin B12.

Formula*

Ingredients	g/L
Dipotassium Phosphate	14.0
Monopotassium Phosphate	6.0
Ammonium Chloride	5.0
Glucose	5.0
DL- Asparagine	3.0
Ammonium Nitrate	2.0
Sodium Chloride	1.0
Magnesium Sulphate	0.2
L- Arginine Hydrochloride	0.2
Ammonium Sulphate	0.1
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	15.0
Final pH (at 25˚C)	7.2 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store below 8°C in tightly closed container, preferably in dessicators and use freshly prepared medium. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 51.50 g of the powder in 1000 mL purified / distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Mix well to distribute slight precipitate evenly.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Cream coloured, homogenous, free flowing powder with a tendency to clump.
Prepared Appearance: Beige to light amber coloured, slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation for 18-24 hours at 30ºC -35°C.

Organism (ATCC)	Growth
Escherichia coli 113-3D (11105)	Good

Note: Microbiological assay of Vitamin B12 is carried out using Escherichia coli mutant 113-3 Davis ATCC 11105 as a test organism and good growth is obtained around cups containing Vitamin B12 showing an increase in diameter of zone of growth in proportion the increasing Vitamin B12 concentration in cup.

Interpretation of 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 more than ±10%.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Care must be taken to avoid contamination of media or glassware in microbiological assay procedures.
2. Detergents or other chemicals present in the glassware may give erroneous results and therefore glassware must be heated to 250ºC for at least 1 hour to burn off any organic residues that might be present.
3. Sterilization and cooling conditions must be kept uniform throughout the assay
4. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
5. All conditions of the assay must be followed precisely as outlined in the references.
6. The use of altered or deficient media may cause mutants having different nutritional requirements and will not give satisfactory response.
7. Over heating or over sterilization will give unsatisfactory results.
8. Sometimes lumps may be formed which will affect the performance of the medium.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. US Pharmacopeial 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.

Product Presentation:

Cat No.	Product description	Pack Size
201020010100	Dehydrated Culture Media	100 g