

## Product Data Sheet

### **B12 ASSAY AGAR (using E.coli mutant Culture)**

**Product No.** GB-DCM-00056-1A

### **INTENDED USE**

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

### **PRODUCT SUMMARY**

B12 Assay Agar 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.

### **Product Specifications**

| <b>Ingredients</b>        | <b>Gms / Ltr</b> |
|---------------------------|------------------|
| 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

The medium contains sucrose and yeast extract which acts as a source of energy. The phosphate ions present buffers the medium. Other salts present helps in maintaining osmotic balance. Agar acts as a solidifying agent.

### INSTRUCTION FOR USE

- Dissolve 51.5 grams in 1000 ml of purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C
- If slight precipitate occurs after autoclaving, distribute it evenly before pouring into sterile Petri plates.

### QUALITY CONTROL SPECIFICATIONS

Appearance of Powder: Off-white to beige homogeneous free flowing powder.  
 Appearance of prepared medium : Yellow coloured, clear to slightly opalescent gel with a slight precipitate forms in Petri plates.  
 pH (at 25°C) : 7.2±0.2

### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 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

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

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

**This product is for research use only.**