B12 Assay Medium (Lactobacillus leichmannii Culture)

Intended Use
B12 Assay Medium (Lactobacillus leichmannii Culture) is used for determining Vitamin B12 concentration by the microbiological assay technique.

Summary
Three types of Vitamin assay media are generally used in the microbiological assay of vitamins:
1. Maintenance Media: For carrying the stock culture to preserve its viability and sensitivity of test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test.
B12 Assay Medium is used for the microbiological assay of vitamin B12 according to USP *Lactobacillus leichmannii* as the test organism.

Principle
Vitamin B12 Assay Medium is a Vitamin B12 free medium containing all other vitamins and nutrients essential for the growth of *E. coli* mutant 113-3D ATCC 11105 and *Lactobacillus leichmannii* ATCC 7830. The growth response obtained is turbidometrically or acidimetrically measured. A standard curve is plotted with absorbance as a function of the vitamin B12 concentration. The concentration of vitamin B12 in the test sample is calculated based on the interpretation of the standard curve.

Formula*

Ingredients	**g/L**
Vitamin Assay Casamino Acids	15.0
Dextrose	40.0
Asparagine	0.2
Sodium Acetate	20.0
Ascorbic Acid	4.0
L-Cystine	0.4
DL-Tryptophan	0.4
Adenine Sulphate	0.02
Guanine Hydrochloride	0.02
Uracil	0.02
Xanthine	0.02
Riboflavin	0.001
Thiamine Hydrochloride	0.001
Biotin	0.00001
Niacin	0.002
p-Aminobenzoic Acid	0.002
Calcium Pantothenate	0.001
Pyridoxine Hydrochloride	0.004
Pyridoxal Hydrochloride	0.004
Pyridoxamine Hydrochloride	0.0008
Folic Acid	0.0002
Monopotassium Phosphate	1.0
Dipotassium Phosphate	1.0
Magnesium Sulphate	0.4
Sodium Chloride	0.02
Ferrous Sulphate	0.02
Manganese Sulphate	0.02
Polysorbate 80	2.0
Final pH (at 25°C)	6.0 ± 0.1

*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.

Type of specimen
Pharmaceutical sample

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 85.00 g of the powder in 1000 mL of purified / distilled water.
2. Heat with frequent agitation and boil for 2-3 minutes to dissolve the powder completely.
3. Dispense in quantities of 5 mL tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL with distilled water.
6. Sterilize by autoclaving at 121°C (15 psi) for 5 minutes as per validated cycle.

Quality Control
Dehydrated Appearance: Cream to yellow coloured, having a tendency to form soft lumps which can be easily broken down to powder form.
Prepared Appearance: Light yellow to amber coloured, clear solution without any precipitate.
Cultural Response: Microbial assay of vitamin B12 is carried out using *Lactobacillus leichmannii* ATCC (7830) after incubating at 30°C-35°C for 16-24 hours.

Organisms (ATCC)	**Growth**
Lactobacillus leichmannii (7830)	Vitamin B_{12} assays passes

Note: For Growth - Gradual increase in growth with increasing USP cyanocobalamin reference standard levels of 0.0, 0.025, 0.050, 0.075, 0.1, 0.125, 0.150 and 0.2 ng per assay tube is recorded as equivalent increase in absorbance at 620 nm.

Interpretation of Results
Refer to USP and other publications for procedures and Interpretation of Results.

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. Ensure that the medium as well as the glassware used in the assay is free from contamination.
2. Heat all glassware at 250°C for at least one hour to burn off any organic residues that might be present before use.
3. Ensure that the sterilization and cooling conditions are uniform throughout the assay.
4. The test organisms used for inoculating an assay must be contained and maintained on media recommended for this purpose.
5. The use of altered or deficient media may give rise to mutants having different nutritional requirements and hence will not give a satisfactory response.
6. Use the aseptic technique throughout the assay procedure.

Warranty

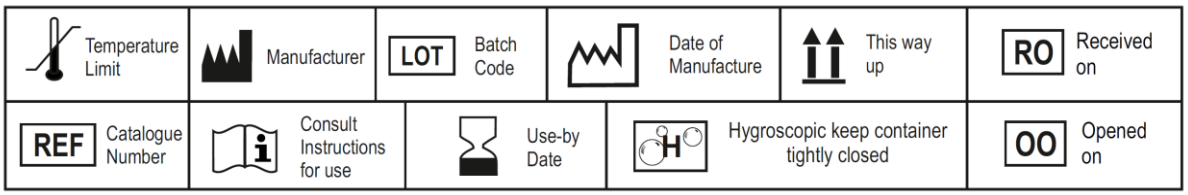
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. II. AOAC International, Gaithersburg, Md.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020020100	Dehydrated Culture Media	100 g



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Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.