



## PARISHIL LABORATORIES PVT.LTD.

3<sup>RD</sup> Floor JivabhaiChembers,OPP. BATA Show Room, Ashram Road,  
AHMEDABAD

**PARTY** :Briyosis

**SAMPLE** : Briyomin

MICROBIAL LIMIT TEST FOR : Vitamin B-12 ( Cyanocobalamine )

METHOD: MICROBIOLOGY Assay Cup plate method

Organism : E.Coli. M-200

Medium : Vitamin B12 Assay Medium – M-110

Sr. NO	Ingredients	Quantity/100ml
1	Disodium hydrogen Phosphate	0.7g
2	Potassium dihydrogen Phosphate	0.3g
3	Sodium Citrate	0.05g
4	Magnesium sulphate	0.01g
5	Ammonium Sulphate	0.1g
6	SodiumChloride	0.005g
7	Distilled Water	100ml

- Dissolve the ingredient separately and mix solution. Adjust pH of the solution to 7.0.
- Add 1.5 gm of agar powder and sterilize at 15lbs pressure for 15min.

Dextrose Solution: Prepare 40% solution w/v solution of Dextrose using D/w.  
Sterilize it coo & store.

Inoculum Medium : Nutrient broth

### Preparation of assay plate:

- To 100ml of sterile cooled Vit B12 Assay medium prepared as above , add 1.0ml 40% Dextrose solution and shake well
- Add 1.0ml of E.coli M200 .Mix thoroughly and distribute 25ml of inoculated medium in sterile petriplates
- Allow to set the medium and store in refrigerator until use.
- Plate sholud be used on the same day or within one day.

### Preparation of Agar cups

A std 8.0 mm diameter is taken. Dip the borer in IPA and sterilise it, cool it and bore cups in the agar plate.

Std. Solution:



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- 20mg. Of cyanocobalamine W .Std dissolve in 20 ml with water. (stock)
- Take 1.0ml and dilute to 100ml. Measure O.D 361 nm.
- Calculate Conc of Stock solution by  
$$\frac{\text{OD at 361nm} \times 100}{0.207} = \text{near } 100 \text{mcg/ml}$$
- From stock 100mcg/ml take 1ml and dilute to 100 ml with water. (1 mcg/ml)
- To 1 ml of this solution and dilute to 100 ml with water.
- To 10 ml of this solution and dilute to 100ml with water.

### Sample Solution:

- Eq. to 10 mcg of cyanocobalamine dissolve in 100 ml with water.
- 5 ml of this solution and dilute to 10 ml with water.

: zone Diameter:

Std High (SH)	Std Low (SL)	Test High (TH)	Test Low (TL)

### Calculation:

% of Potency = Antilog  $2 + (a \log I)$

$$a = \frac{(TH+TL) - (SH+SL)}{(TH - TL) + (SH-SL)}$$

Log I = 0.3010

Test High (TH) Std High (SH)

Test Low (TL) Std Low (SL)

Note : Dilutions according to label claim.