

# VITAMIN B12 QUANTI MICROLISA

Microwell ELISA Immunoassay for the Quantitative Detection of vitamin B12 (cyanocobalamin) in Human Serum

## 1. INTRODUCTION

Vitamin B12, also called cyanocobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system, and the formation of red blood cells. It is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism. Vitamin B12 is essential for the manufacture of red blood cells; a deficiency leads to a characteristic kind of anemia.

Vitamin B12 is bound to proteins in food and once it reaches the gut, it is released by the acids present. The B12 then binds the intrinsic factor (IF). Vitamin B12 in bound form is stable and travels into the intestine where it can be absorbed in the body through its association with IF.

Vitamin B12 deficiency is most commonly caused by low intakes, but can also result from malabsorption, certain intestinal disorders, low presence of binding proteins, and use of certain medications. Vitamin B12 is rare from plant sources, so vegetarians are most likely to suffer from vitamin B12 deficiency. Infants are at a higher risk of vitamin B12 deficiency if they were born to vegetarian mothers. The elderly who have diets with limited meat or animal products are vulnerable populations as well.

Vitamin B12 deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, depression, and poor memory may be experienced. Vitamin B12 deficiency can also cause symptoms of mania and psychosis.

## 2. INTENDED USE














Vitamin B12 Quanti Microlisa is designed for in-vitro quantitative determination of Vitamin B12 in human serum.

## 3. PRINCIPLE

Vitamin B12 Quanti Microlisa is an enzyme immuno assay based on competitive ELISA. First samples have to be pre-treated and in separate glass test tubes with working denaturation reagent to extract the vitamin B12 as circulating B12 is bound to binding protein in-vivo. After denaturation and neutralization, treated sample is added in the microwells followed by addition of Anti- vitamin B12 biotin conjugate. During incubation, endogenous vitamin B12 of a sample binds with the Anti- vitamin B12 biotinylated conjugate. After a short incubation, Vitamin B12 Enzyme Reagent (peroxidase labelled vitamin B12) is added. (This delayed competition increases the sensitivity for low concentration Vitamin B12 samples). During this incubation, endogenous vitamin B12 of a sample competes with the vitamin B12 Enzyme Reagent for binding of anti-vitamin B12 antibodies & simultaneously reaction occurs between the Biotin conjugated to Anti-Vitamin B12 and Streptavidin immobilized on microwells. Incubation is followed by a washing step to remove unbound components. In the third incubation, color reaction is started by addition of substrate & its binding with Vitamin B12 Enzyme reagent and stopped after a defined time. The color intensity is inversely proportional to the concentration of Vitamin B12 in the sample.

## 4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.

 Manufactured By	 <i>In vitro</i> diagnostic medical device
 No. of tests	 See Instruction for use
 Lot Number / Batch Number	 Temperature Limitation
 Manufacturing Date	 Caution See instruction for use
 Expiry Date	 Catalogue Number
 Do not use if package is damaged	 Keep away from sunlight
 Keep Dry	

## 5. PACK SIZE

- 96 Tests

## 6. COMPONENTS IN EACH Vitamin B12 Quanti Microlisa Kit (96 Tests)

Store all components at 2-8°C when not in use. Expiry date on the kit indicates that beyond which the kit should not be used.

<b>Vitamin B12 Quanti Strip Plates</b>	12 Strips (96 wells) Breakway microwells coated with streptavidin packed in a pouch with desiccant.
<b>Denaturation Reagent</b>	1 Bottle Sodium hydroxide solution.
<b>Stabilizing Reagent (41x)</b>	1 Vial Reducing agent solution.
<b>Neutralization Reagent</b>	1 Bottle, Ready to use Buffer solution containing preservative.
<b>Anti-Vitamin B12 Biotin Conjugate</b>	1 Vial, Ready to use Containing anti-vitamin B12 conjugated to biotin, with preservatives.
<b>Vitamin B12 Enzyme Reagent</b>	1 Vial, (Ready to use) Containing peroxidase conjugated to vitamin B12, with preservatives.
<b>Wash Buffer Concentrate (25x)</b>	1 Bottle PBS with surfactant. Dilute 1:25 with distilled water before use.
<b>TMB Substrate</b>	1 Bottle To be diluted with TMB diluent before use.
<b>TMB Diluent</b>	1 Bottle Buffer solution containing H <sub>2</sub> O <sub>2</sub> with preservative
<b>Calibrator-1</b>	1 Vial: 0 pg/ml of vitamin B12 in Human Serum and containing preservatives.
<b>Calibrator-2</b>	1 Vial: 100 pg/ml of vitamin B12 in Human Serum and containing preservatives.
<b>Calibrator-3</b>	1 Vial: 200 pg/ml of vitamin B12 in Human Serum and containing preservatives.
<b>Calibrator-4</b>	1 Vial: 400 pg/ml of vitamin B12 in Human Serum and containing preservatives.
<b>Calibrator-5</b>	1 Vial: 1000 pg/ml of vitamin B12 in Human Serum and containing preservatives.
<b>Calibrator-6</b>	1 Vial: 2000 pg/ml of vitamin B12 in Human Serum and containing preservatives.
<b>Stop Solution</b>	1 Bottle, Ready to use 1N sulfuric acid
<b>Plate Sealers</b>	Adhesive backed sheets for sealing microwell plate/strips.

## 7. STORAGE AND STABILITY

Store the kit & its component at 2-8°C. Expiry date on the kit indicates the date beyond which kit should not be used, open reagents are stable for 60 days when store at 2-8°C.

## 8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- ELISA Reader
- Distilled or deionized water
- Disinfectant solution
- Glass test tube
- Paper towels or absorbent tissue
- Timer
- Microplate washer
- Incubator 37°C
- Vortex Mixer
- Disposable gloves

## 9. SPECIMEN COLLECTION & PREPARATION

1. Only human serum should be used for the test. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis and stored in dark being light sensitive. Fresh serum samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for three days, or frozen at -20°C or lower for 15 days.
3. Use of heat inactivated, icteric hyperlipemic and hemolyzed samples should be avoided as may give erroneous results.

## 10. SPECIMEN PROCESSING

### (A) FROZEN SAMPLE

Vitamin B12 Quanti Microlisa test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed. Only 1 thawing of sample is allowed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged. (10,000 rpm for 15 min.)

### (B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

## 11. WARNING & PRECAUTION



**CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. LOW CONTROL, HIGH CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves is RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be disposed off in accordance with established safety procedures and guidelines.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

## 12. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Bring all the reagents & samples to room temperature (20-30°C) before use.
3. Do not combine reagents from different batches, as they are optimised for individual batch to give best results.
4. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
5. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
6. Use freshly collected, clean serum samples for assay. Try to avoid turbid, lipemic serum samples.
7. Use a separate tip for each sample and then discard it as biohazardous waste.
8. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
9. Do not allow microwells to dry once the assay has started.
10. Run all six calibrators, in each assay to evaluate validity of the kit.

11. Incubation time should not vary by more than  $\pm 2$  min.
12. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
13. Distilled or deionised water must be used for wash buffer preparation.
14. Thorough washing of the wells is critical to the performance of the assay. Overflowing of reagents or washing to adjacent wells must be prevented during washing, which may lead to incorrect results due to carry over effect.
15. Take care while preparing working substrate solution as Bottle of TMB Substrate & TMB Diluent are of same size.
16. Prepare working substrate solution just 10 minutes prior to adding in the wells.
17. Use separate tips for TMB Substrate and TMB diluent.
18. Avoid strong light exposure during the assay.
19. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
20. In case of any doubt the run should be repeated.

## 13. PREPARATION OF REAGENTS

Prepare the following reagents just before or during assay procedure. Reagents and samples should be at room temperature (20-30°C) before beginning the assay. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre-warm the incubator at 37°C.

### 13.1 Vitamin B12 Quanti Microlisa strips :

Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the microwell strips.

- a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that six calibrators should be included in first run and atleast three calibrators (0, 400 & 2000 pg/ml) in subsequent runs.
- b. **Unused wells should be stored at 2-8°C, with dessicant in an aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with desiccant along with clamp & rod.**

**Caution:** Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

### 13.2 Preparation of working Denaturation Reagent

Mix Denaturation reagent and Stabilizing Reagent (41x) to prepare working Denaturation Reagent as given below:

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
Stabilizing Reagent 41x ( $\mu$ l)	25	50	75	100	125	150	175	200	225	250	275	300
Denaturation Reagent (ml)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0

**Do not store working Denaturation Reagent.**

### 13.3 Preparation of Wash Buffer:

- a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.
- b) Prepare at least 25 ml (1.0 ml concentrated buffer with 24.0 ml water) of buffer for each ELISA strip used. Mix well before use.
- c) Mix 20 ml of 25x wash buffer concentrate with 480 ml. of distilled or deionized water. Wash buffer is stable for 2 months when stored at 2-8°C.

### ● Preparation of working substrate solution :

Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6
TMB Diluent (ml)	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6

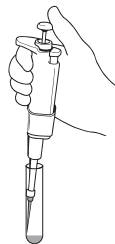
**Do not store working substrate.** Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

#### 14. PROCEDURAL NOTES:

1. Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-30°C) before testing begins. Return the reagents to 2-8°C after use.
2. All reagents must be mixed well before use.
3. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
4. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.

**Note: Touch side of tube with pipette while dispensing samples and reagents at an angle near the bottom of the tube.**

5. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
6. In case precipitation is observed then it could be due to present of high amount of proteins in the samples, such samples should be diluted 1:1 with a normal saline before treatment of samples.



#### 15. TEST PROCEDURE

**The instructions of the procedure must be strictly followed.**

The sequence of the procedure must be carefully followed. Arrange the calibrators in a horizontal or vertical configuration. Configuration is dependent upon reader software. It is recommended to perform all six calibrators to run and calculate the results using master curve.

##### 15.1 Sample Treatment

- i) Add 100 µl of each calibrator and samples in neat and clean individual glass test tube.
- ii) Add 50µl of working denaturation reagent prepared in (Step-13.2) in above sample/ calibrator tube and mix properly on a vortex mixer for 1-2 seconds.
- iii) Incubate the tubes at R.T. for 15 minutes.
- iv) Add 100µl Neutralization Reagent and mix immediately on a vortex mixer.
- v) Incubate at R.T. for 5 minutes.

**Note: Mixing of sample on vortex mixer should be done immediately before moving to next tube/sample.**

##### 15.2 ELISA Procedure

- i) Secure the required no. of appropriate coated microwells for the assay.
- ii) Transfer 100µl of treated calibrators and sample into the respective microwells.
- iii) Add 25 µl of anti-vitamin B12 Biotin conjugate into each well. Thoroughly mix for 10-20 seconds.
- iv) Cover the microwell plate & incubate at 37°C for 60 minutes.
- v) Add 25 µl of Vitamin B12 Enzyme Reagent into each well. Thoroughly mix for 10-20 seconds
- vi) Cover the microwell plate & incubate at 37°C for 30 minutes.
- vii) Dilute the wash buffer concentrate with distilled water to 1:25 dilution.
- viii) At the end of incubation period, take out the plate from incubator.
- ix) Wash with working wash buffer.

**WASHING:** Washing can be performed either with WASHER or manually as follows:

- a). Empty the wells.
- b). Add 300-350µl of working washing solution into each well.
- c). Empty the wells.
- d). Wash each well 3 times in total.
- e). After the third wash, tap dry the Microwells a few times on an absorbent tissue.
- x) Add 150 µl working substrate solution in each well.
- xi) Incubate at R.T. in dark for 15 mins. and do not expose to light.
- xii) Add 50 µl of stop solution to each well.
- xiii) Read the absorbance at 450 & 630 nm within 10 minutes in ELISA reader.

#### 16. CALCULATION OF RESULTS

1. Construct a calibrator master curve by plotting the absorbance obtained from each calibrator against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis as log (X) - logit (Y) by competitive method.
3. Using the absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated Method : The results have been calculated automatically using curve regression mode which is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the sample can be read directly from the standard curve. Sample with concentrations higher than that of the highest standard have to be further diluted (1:2 or 1:5) or reported as > 2000 pg/ml. For the calculation of the concentration, this dilution factor has to be taken into account.
6. For subsequent run, once master curve has been established in an ELISA Reader, calculate the results with stored master curve and absorbance of 3 calibrators with necessary data analytics.

**Important Note: QC data sheet is batch specific and can be downloaded from company web site; [www.jmitra.co.in](http://www.jmitra.co.in)**

#### 17. EXPECTED VALUES

Each laboratory should establish its own range of normal value. The values given below are only indicative.

Population	Vitamin B12 (pg/mL)	Vitamin B12 (pmol/L)
New Born	160-1300	118-959
Adult	200-835	148-616
Adult > 60 years	110-800	81-590

Concentration of samples can be expressed in pmol/L by multiplying by 0.738. e.g. 100 pg/ml x 0.738 = 73.8 pmol/L.

#### 18. PERFORMANCE CHARACTERISTICS

##### 18.1 Linearity:

Vitamin B12 Quanti Microlisa is linear between 50 pg/ml to 2000 pg/ml.

##### 18.2 Specificity of antibodies (Cross Reactivity):

The specificity of the Vitamin B12 Quanti Microlisa is tested with potentially interfering substance like Rheumatoid factor, cobinamide, Haemoglobin (upto 500 mg/dl), Bilirubin (upto 40 mg/dl) and Triglycerides (upto 500 mg/dl) and these substances have no influence on the ELISA results.

##### 18.3 Detection Limit:

The detection limit of the Vitamin B12 Quanti Microlisa is 50 pg/mL.

##### 18.4 Accuracy:

The accuracy of Vitamin B12 Quanti Microlisa is checked with 10 clinical specimen and compared with reference ELISA test kit. The co-relation co-efficient is > 0.99.

##### 18.5 Reproducibility:

###### 18.5.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (pg/mL)	CV(%)
1	10	92.6	9.92
2	10	662.1	6.01
3	10	2240	5.33

###### 18.5.2 Inter Assay

The reproducibility between assay is shown below:

Sample	n	Mean (pg/mL)	CV(%)
1	10	93.6	14.3
2	10	710.7	6.16
3	10	2381.2	7.0

#### 19. LIMITATION OF THE TEST

1. Any improper handling of samples or modification of this test might influence the results.
2. Samples which show turbidity, hemolysis, hyperlipemia high protein or contain fibrin may give erroneous results.
3. No hook effect was observed in this test
4. No substances (drugs) are known to us, which have an influence to the measurement of Vitamin B12 in a sample.

## 20. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

## 21. REFERENCES

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2. Lee DSC, Griffiths BW. human serum vitamin B12 Assay method, a review. Clin. Biochem 1985 - 18:261-266.
3. Charain L. megablolastic Anemia, cobalmin and folate. J. Clin, pathol, 1987 40:978-984.
4. Klee GC, Cobalmin and folate evaluation: measurement of methyl malonic acid and homocysteine vs vitamin B12 and folate clin. chem. 46; 2000:1277-83.
5. Annals of clinical and laboratory science Vol. 27 No.4, 1997, 249-53.

## 22. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Standards curve out of validation limit	a) Incorrect temperature timing or pipetting	Check procedure & repeat assay
	b) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
	c) Cross contamination of standards/ reagents	Pipette carefully and do not interchange caps. use separate tip for standards / reagents.
	d) Used components from different lots.	Do not use components from different lots as they are adjusted for each batch released.
	e) Expired Reagents	Check the kit expiry date. Use the kit with-in shelf life
	f) Use of non calibrated micropipette and/or ELISA Reader	Calibrate micropipette and ELISA Reader at defined interval.
2. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of anti-vitamin B12 Biotin conjugate and Vitamin B12 Enzyme Reagent.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect temperature timing or pipetting	Check procedure & repeat assay
	g) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.

PROBLEM	POSSIBLE CAUSE	SOLUTION
4. Poor reproducibility	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of glass distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing.	Check wash device, fill the well close to the top.
	i) Washing not consistent	After washing, blot the microwells on absorbent tissue. Follow wash protocol meticulously
	ii) Filling volume not sufficient.	
	iii) Insufficient no. of wash cycles.	
	iv) Contaminated wash device	
	e) Use of wash solution from other manufacturer.	Use only Vitamin B12 Quanti Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
	a) Washing problems.	
5. High O.D for Standards & Samples	b) Uncalibrated pipettes or tips not well fitted, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Reagent & sera not at room temperature or not well mixed before use.	Equilibrate reagents to room temperature and mix thoroughly before use
	d) Too long time for addition of calibrators, samples or reagents. Inconsistency in time intervals.	Develop consistent and uniform technique.
	e) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
6. Low O.D for Standards and samples	Beside 3a, b, c, d, e, incorrect interpretation and calculation of final results.	Check the calculation part given in the instruction manual and correctly interpret.
	a) Inadequate addition of standards/ substrate/conjugate solution	Follow the test procedure meticulously & repeat assay.
7. High O.D for Standards and samples	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of anti-Vitamin B12 Biotin conjugate and/ or Vitamin B12 Enzyme Reagent.	Check storage of anti- Vitamin B12 Biotin conjugate and Vitamin B12 Enzyme Reagent. It shall be stored at 2-8°C.
	f) Stop solution is added before 15 minutes. Reaction terminated before 15 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.

*in vitro* diagnostic Reagent, not for medicinal use

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