AIA-600II Assay Specifications
B12 Test Code 018

Screen	Item	Data
Screen 1		
	Lot	***Enter current cal. lot no.
	Cal 1	0 pg/mL
	Cal 2	100 pg/mL (example.)
	Cal 3	250 pg/mL (example.)
	Cal 4	500 pg/mL (example.)
	Cal 5	1000 pg/mL (example.)
	Cal 6	2100 pg/mL (example.)
Screen 2		
	Name	B12
	Unit	pg/mL
	Smpl	100
	Dil	50
	2Reag	0
	Code	18
	Assay Range Low	50
	Assay Range High	2000
	Reference Range Low	
	Reference Range High	
	DP (No. of decimal points)	0
Screen 3		
	Code	18
	No. (Calibrators)	6
	Mul. (Replicates)	3
	Equ	6
	CV (Calibration curve stability)	30
	STAT (Analyte status)	0
	PRCL (Assay Protocol)	2
Screen 4		
	Dilution Factors:	
	SP1 (Specimen 1)	1
	SP2 (Specimen 2)	1
	CAL	1
	CTRL	1
	CODE (SDS code)	18
	PR (Dilution mode)	3
	Pretreatment:	
	SMPL (Pretreatment sample volume)	200
	VOL1 (Pretreatment 1 volume)	100
	VOL2 (Pretreatment 2 volume)	100
	CODE (Pretreatment code)	18

AIA-900 Assay Specifications
B12 Test Code 018

No.	Item	Data
1	Code	18
2	ACT	0
3	Analyte	B12
4	Lot	***Enter current cal. lot no.
5	CAL 1	0 pg/mL
6	CAL 2	100 pg/mL (example.)
7	CAL 3	250 pg/mL (example.)
8	CAL 4	500 pg/mL (example.)
9	CAL 5	1000 pg/mL (example.)
10	CAL 6	2100 pg/mL (example.)
11	Cal lot L	
12	Cal lot R	
13	Unit	pg/mL
14	Decimal	0
15	Assay Low	50
16	Assay High	2000
17	Reference Low	
18	Reference High	
19	Reschedule Low	50
20	Reschedule High	2000
21	Factor A	1
22	Factor B	0
23	Sample Volume	100
24	Diluent Volume	50
25	2 Step reagent dispensing volume	0
26	Calibration code	18
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	6
30	CAL. CV	
31	DIL. SP1	1
32	DIL. SP2	1
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	1
35	DIL. DO	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	18
39	DIL. NAME	B12
40	DIL. PRTY	3
41	PRE. SPVOL (Vol. of pretreated sample)	200
42	PRE. 1VOL (Vol. of pretreatment sol-1)	100
43	PRE. 2 VOL (Vol. of pretreatment sol-2)	100
44	PRE. CODE (pretreated sol. code)	18
45	PRE. NAME (pretreated sol. name)	B12
46	Protocol	2
47	SYS. F_A	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-1800 Assay Specifications
B12 Test Code 018

No.	Item	Data
1	Unit	pg/mL
2	Decimal places	0
3	Reference low	0
4	Reference high	0
5	Reschedule low	50
6	Reschedule high	2000
7	Assay range low	50
8	Assay range high	2000
9	Specimen diluent code	018
10	Specimen diluent name	B12
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	100
15	Default multiplier for >H	10
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	006
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01 2001800101960001	0 pg/mL
23	Cal - 02 2001800102960001	100 pg/mL (example.)
24	Cal - 03 2001800103960001	250 pg/mL (example.)
25	Cal - 04 2001800104960001	500 pg/mL (example.)
26	Cal - 05 2001800105960001	1000 pg/mL (example.)
27	Cal - 06 2001800106960001	2100 pg/mL (example.)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	30
36	Incubation time	40
37	Assay protocol	2
38	Specimen volume	100
39	Diluent volume	50
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	1
43	Pretreatment specimen volume	200
44	Pretreatment Reagent code	18
45	Pretreatment Reagent name	B12
46	Pretreatment Reagent 1 volume	100
47	Pretreatment Reagent 2 volume	100
48	System Factor	1
49	Calibration Code Check	018
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

AIA-2000 Assay Specifications
B12 Test Code 018

No.	Item	Data
1	Unit	pg/mL
2	Decimal places	0
3	Reference low	0
4	Reference high	0
5	Reschedule low	50
6	Reschedule high	2000
7	Assay range low	50
8	Assay range high	2000
9	Specimen diluent code	018
10	Specimen diluent name	B12
11	Dilution factor for Sp. 1	1
12	Dilution factor for Sp. 2	1
13	Dilution factor for Control	1
14	Default multiplier for DO	100
15	Default multiplier for >H	10
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	006
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01 2001800101960001	0 pg/mL
23	Cal - 02 2001800102960001	100 pg/mL (example.)
24	Cal - 03 2001800103960001	250 pg/mL (example.)
25	Cal - 04 2001800104960001	500 pg/mL (example.)
26	Cal - 05 2001800105960001	1000 pg/mL (example.)
27	Cal - 06 2001800106960001	2100 pg/mL (example.)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	30
36	Incubation time	40
37	Assay protocol	2
38	Specimen volume	100
39	Diluent volume	50
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	1
43	Pretreatment specimen volume	200
44	Pretreatment Reagent code	18
45	Pretreatment Reagent name	B12
46	Pretreatment Reagent 1 volume	100
47	Pretreatment Reagent 2 volume	100
48	System Factor	1
49	Calibration Code Check	018
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Vitamin B12
AIA-PACK B12

Name and Intended Use

AIA-PACK B12 is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of vitamin B12 (B12) in human serum on Tosoh AIA System analyzers.

Summary and Explanation of Test

Synthesized exclusively by micro-organisms and found in nearly all animal tissues, vitamin B12 (cyanocobalamin) is essential for metabolism of fatty acids, intermediary metabolism of folic acid, and the biosynthesis of DNA and RNA. After ingestion and initial protein digestion, the vitamin B12 forms complexes with the gastric glycoprotein carriers, intrinsic factor (IF) and R factors. During digestion, the B12-IF complex is bound by receptors in the terminal ileal mucosa. The complex is dissociated as the vitamin B12 enters capillary to portal circulation for storage in the liver. Bound to the transport protein, transcobalamin II, vitamin B12 is released by hepatocytes, as physiological needs arise.

Deficiency of vitamin B12 may be produced by any of several factors, including inadequate dietary intake and malabsorption. Malabsorption, the most common cause of vitamin B12 deficiency, is associated with auto-immune disorders (pernicious anemia), gastric disorders, and damage to or absence of the terminal ileum.

Elevated levels of vitamin B12 are most commonly caused by ingestion of vitamin supplements. Elevated levels of vitamin B12, which persist after cessation of vitamin supplements, may indicate liver damage or myelopoliferative diseases.

Vitamin B12 deficiency can produce serious, progressive and if untreated, often irreversible neurological disorders and macrocytic or megaloblastic anemia. Because morphologically indistinguishable megaloblastic anemias may be caused by deficiencies in either vitamin B12 or folic acid, it is important to determine serum levels of both to properly diagnose the cause and effectively treat the anemia. [1-6]

Principle of the Assay

The AIA-PACK B12 is a competitive enzyme immunoassay which, after sample pretreatment, is performed entirely within the AIA-PACK. Sample pretreatment reagents (containing potassium cyanide, sodium hydroxide, and dithiothreitol) release vitamin B12 from serum binding proteins in the sample and converts cyanocobalamin into a stable, measurable form of vitamin B12. Vitamin B12 present in the pretreated test sample competes with enzyme-labeled vitamin B12 for a limited number of binding sites on a fluorescein labeled porcine intrinsic factor which then binds to anti-FITC antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme labeled vitamin B12 and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme labeled vitamin B12 that binds to the beads is inversely proportional to the vitamin B12 concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown vitamin B12 concentrations are calculated using this curve.

Material Provided (AIA-PACK B12, Cat. No. 020293)

10 trays x 20 test cups (AIA-PACK B12 Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with anti-FITC mouse monoclonal antibody and FITC labeled porcine intrinsic factor and vitamin B12 conjugated to bovine alkaline phosphatase with 0.1% sodium azide as a preservative.

Materials Required But Not Provided

The following materials are not provided but are required to perform vitamin B12 analysis using the AIA-PACK B12 (Cat. No.020293) on the Tosoh AIA Systems. They are available separately from Tosoh.

Materials	Cat. No.
AIA-SYSTEMS:	
AIA-600II	019014
AIA-600II BCR	019328
AIA-900	022930
AIA-900 9tray Sorter	022931
AIA-900 19tray Sorter	022932
AIA-1800 ST	019836
AIA-1800 LA	019837
AIA-2000 ST	022100
AIA-2000 LA	022101
AIA-PACK:	
AIA-PACK SUBSTRATE SET II	020968
AIA-PACK SUBSTRATE/RECONSTITUENT	
AIA-PACK B12 PRETREATMENT SET Pretreament-1 / PRETREATMENT-2 / PRETREATMENT-3	020706
AIA-PACK B12 CALIBRATOR SET	020393
CALIBRATOR #1 0 pg/mL	
CALIBRATOR #2 100 pg/mL (approx.)	
CALIBRATOR #3 250 pg/mL (approx.)	
CALIBRATOR #4 500 pg/mL (approx.)	
CALIBRATOR #5 1000 pg/mL (approx.)	
CALIBRATOR #6 2100 pg/mL (approx.)	
AIA-PACK B12 SAMPLE DILUTING SOLUTION	020593
AIA-PACK WASH CONCENTRATE SET	020955
AIA-PACK DILUENT CONCENTRATE SET	020956
AIA-PACK DETECTOR STANDARDIZATION TEST CUPS	020970
AIA-PACK SAMPLE TREATMENT CUPS	020971
Sample Cups	018581
ADDITIONAL REQUIREMENTS:	
PIPETTE TIPS (1000/PKG)	019215
TIP RACK (EMPTY)	019216
PRELOADED PIPETTE TIPS (96 TIPS X 50 RACKS)	996010
PRELOADED PIPETTE TIPS (96 TIPS X 5 RACKS)	996005

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is based strictly on Tosoh materials.

Warnings and Precautions

- The AIA-PACK B12 is intended for in vitro diagnostic use only.
- The AIA-PACK B12 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera is not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
- Do not use beyond the expiration date.
- The AIA-PACK B12 Pretreatment Set used in conjunction with the AIA-PACK B12 assay contains 0.06% potassium cyanide and 1.6% sodium hydroxide. Avoid contact with eyes, skin or clothing. Wash thoroughly after handling. If discarded into drain, flush with a large volume of water.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
Refrigerator Temperature (2° - 8° C):	
AIA-PACK B12	020293
AIA-PACK B12 Calibrator Set	020393
AIA-PACK B12 Sample Diluting Solution	020593
AIA-PACK B12 Pretreatment Set	020706
AIA-PACK Substrate Set II	020968
AIA-PACK Wash Concentrate	020955
AIA-PACK Diluent Concentrate	020956
Room Temperature (18° - 25° C):	
AIA-PACK Detector Standardization Test Cups	020970
AIA-PACK Sample Treatment Cups	020971

AIA-PACK test cups may be stored for up to 24 hours at a room temperature of 18° - 25° C. Calibrators must be kept tightly sealed and refrigerated at 2° - 8° C. After opening, calibrators should be used within 24 hours. Reconstituted Pretreatment-1 should be used within 24 hours. After opening, Sample Diluting Solution is stable for up to 7 days refrigerated at 2° - 8° C. Reconstituted substrate solution is stable for 3 days at room temperature (18° - 25° C) or 30 days in the refrigerator (2° - 8° C). Working diluent and wash solutions are stable for 30 days at room temperature (18° - 25° C). Reagents should not be used if they appear cloudy or discolored.

Specimen Collection and Handling

Serum is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.

No special patient preparation is necessary. A venous blood sample is collected aseptically without additives (Red top tube). Store at room temperature until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay. SST or gel tubes have not been validated.

Samples may be stored at 2° - 8° C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20° C or below for up to 60 days.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to room temperature (18° - 25° C) and mix gently.

All samples require pretreatment. Pretreated samples should be assayed within one hour after completion of the pretreatment procedure.

The pretreated sample required for analysis is 100 µL.

Procedure

1) Reagent Preparation

1a) Substrate Solution

Bring all reagents to room temperature (18° - 25° C) before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate and mix thoroughly to dissolve the solid material.

1b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water, mix well, and adjust the final volume to 2.5 L.

1c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water, mix well, and adjust the final volume to 5.0 L.

1d) Pretreatment Reagents

Bring all pretreatment reagents to room temperature (18° - 25° C). Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to a volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve. Pretreatment-3 is provided ready to use.

2) Pretreatment

2a) Pretreatment Procedure

All samples (human serum, controls, calibrators) must be pretreated prior to assaying on the AIA Systems. On the AIA-System, the pretreatment is performed automatically using the parameters defined in Assay Specifications. If manual pretreatment is desired, follow the instructions for Manual Procedure. The pretreated samples should be assayed within 1 hour after completion of the pretreatment procedure.

2b) Automatic Pretreatment Procedure

i) Bring all pretreatment reagents and samples to room temperature.

ii) Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to the volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve.

iii) Place reconstituted Pretreatment -1 (PT-1) and Pretreatment -3 (PT-3) in the designated positions on the reagent cassette as defined under REAGENT REGISTRATION. Note: On the AIA-600 II, Pretreatment-3 is placed in the position designated as PRETREATMENT (2).

iv) Place the appropriate amount of sample cups/primary tubes, sample treatment cups and AIA-PACK B12 test cups on the instrument.

2c) Manual Pretreatment Procedure

i) Set all pretreatment parameters in the software to 0 (zero).

ii) Bring all pretreatment reagents and samples to room temperature.

iii) Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to the volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve.

iv) Pipette 200 μL of sample (serum, control, calibrator) into a glass or polypropylene tube.

v) Add 100 μL of the reconstituted Pretreatment-1 to each tube and mix well using a vortex mixer.

vi) Incubate the treated sample for 15 minutes at room temperature in the dark.

vii) Add 100 μL of the Pretreatment-3 to each treated sample. Mix well using a vortex mixer.

viii) Samples should be assayed within 1 hour.

3) Calibration

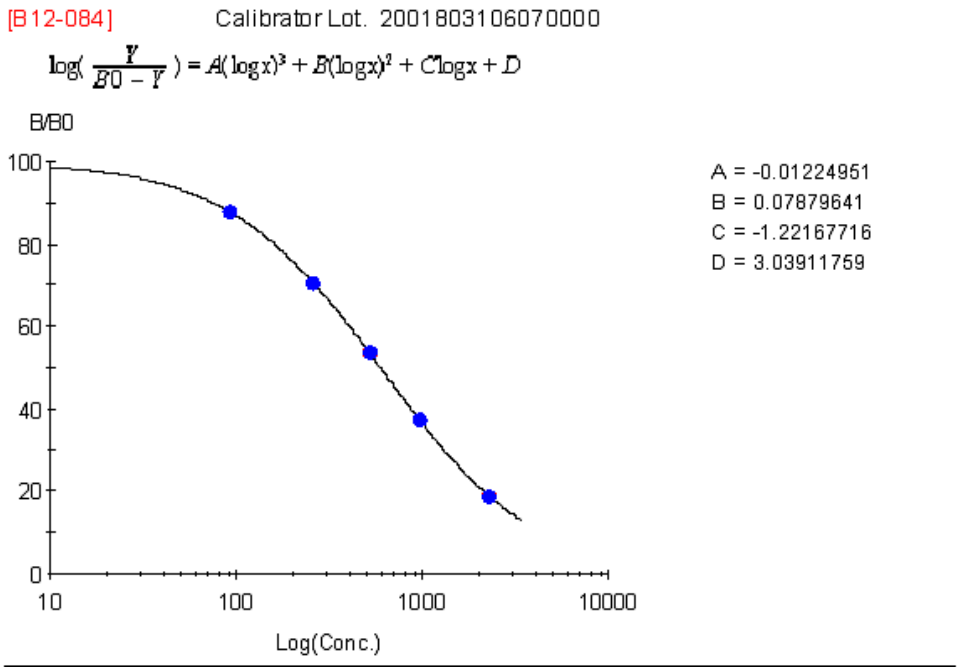
3a) Calibration Curve

The calibrators for use with the AIA-PACK B12 are prepared gravimetrically and are compared to internal reference standards.

The calibration curve for the AIA-PACK B12 is stable for up to 30 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or if certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, or detector lamp adjustment or change). Recalibration is necessary if there is a change in the lot number of the B12 Pretreatment Set. For further information regarding instrument operation, consult the AIA System Operator's Manual.

The sample calibration curve below shows the algorithm used to calculate the results. This is an example only. Actual results will vary depending on the Instrument type and lot number used.



3b) Calibration Procedure

i) Refer to the appropriate AIA System Operator's Manual for procedural instructions.

ii) Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.

iii) Calibrators for AIA-PACK B12 are lyophilized with the exception of the zero calibrator. All other levels should be reconstituted with 1 mL of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water. Tosoh recommends that all calibrators be run in triplicate.

iv) B12 calibrators require pre-treatment. Refer to the pretreatment procedure.

3c) Calibration Acceptability criteria

i) Since there is an inverse relationship between concentration and rate, the rates should decrease as the concentration increases.

ii) The replicate values should be within a 10% range.

3d) Calibration Review and Acceptance

i) Using the criteria above, review the calibration curve carefully.

ii) Edit the calibration if necessary and then accept the calibration.

iii) For further information regarding calibration, consult the specific AIA System Operator's Manual.

4) Quality Control

4a) Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two (2) levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material: _____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

4b) Quality Control Procedure

i) Assay quality control specimens as instructed in the specific Operator's Manual for your analyzer. In addition, refer to the AIA System Operator's Manual for detailed instructions on defining and editing the files.

ii) Quality control material to be run with this assay is defined by individual laboratory policy.

5) Specimen Processing

5a) Preparation

Following specific instructions in the Operator's Manual for your analyzer. Place samples on the instrument appropriately.

5b) Assay Procedure

i) Ensure a sufficient quantity of AIA-PACK B12 test cups for the number of samples to be run.

ii) Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA-600II, AIA-900, AIA-1800 and AIA-2000 will require AIA-PACK Sample Treatment Cups if onboard dilutions are utilized.

iii) Refer to the pre-treatment procedure for automatic and manual pre-treatment.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA Systems require that the laboratory use water designated by the College of American Pathologists as Class I or by NCCLS (CLSI) as Type I. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the NCCLS (CLSI) document "Preparation and Testing of Reagent Water in the Clinical Laboratory," NCCLS (CLSI) Document C3-A2, Volume 11 No. 13, originally approved as a guideline by NCCLS in August 1991.
3. If a serum specimen vitamin B12 concentration is found to be greater than the linearity limit of the assay, 2000 pg/mL; the specimen should be diluted with the AIA-PACK B12 Sample Diluting Solution and re-assayed according to the Assay Procedure.
4. The recommended dilution for samples containing greater than 2000 pg/mL is 1:10. It is desirable to dilute the serum sample so that the diluted sample reads between 250 and 1500 pg/mL. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the AIA System Operator's Manual.
5. The AIA systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of AIA-PACK B12 Test cups can be used during the same run.
6. If the assay specifications for this test are not already in the system software, the specifications must be entered under test code 018.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to vitamin B12 concentration in pg/mL.

For samples requiring dilution, the AIA System will automatically perform dilutions and calculate results if the dilution factors are entered into the software. Dilution factors may be entered when programming the specimens.

Evaluation of Results

Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples be assayed daily.

The minimum recommendations for the frequency of running internal control material are:

- After calibration, three levels of controls are run in order to accept the calibration curve.
- The three levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).
- After daily maintenance, at least two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzers.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

Limitations of the Procedure

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g. symptoms, results of other tests, clinical impressions, therapy, etc.).

Using AIA-PACK B12, the highest concentration of vitamin B12 measurable without dilution is approximately 2000 pg/mL, and the lowest measurable concentration is 50 pg/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 2100 pg/mL, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 2000 pg/mL.

Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.

Expected Values

Each laboratory should determine a reference interval which corresponds to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.[7]

Reference Ranges

Reference Range = 230 - 1050 pg/mL (170 - 775 pmol/L)

Conversion Factors

Vitamin B12 concentrations in this application are in units of pg/mL. Conversion to SI units of pmol/L may be made using the following equation:

pmol B12/L = pg B12/mL x 0.738

Performance Characteristics

The following performance characteristics were determined using the Tosoh AIA-1200 Automated Immunoassay Analyzer. The AIA-Systems demonstrate equivalent performance.

1) Accuracy

1a) Recovery:

Three serum pools were spiked with three different levels of vitamin B12 and assayed before and after spiking.

Sample	Initial Value (pg/mL)	B12 Added (pg/mL)	Expected Value (pg/mL)	Measured Value (pg/mL)	Percent Recovery (%)
Serum A	262.5	202.5	465.0	440.1	94.7
	262.5	404.9	667.4	619.2	92.8
	262.5	809.9	1072.4	933.1	87.0
Serum B	347.5	132.2	479.8	480.7	100.2
	347.5	264.5	612.0	630.9	103.1
	347.5	529.0	876.5	895.3	102.1
Serum C	328.5	177.8	506.3	524.6	103.6
	328.5	355.6	684.0	656.1	95.9
	328.5	711.1	1039.6	928.8	89.3

1b) Dilution:

Three serum samples containing high concentrations of vitamin B12 were serially diluted with AIA-PACK B12 Sample Diluting Solution and assayed.

Sample	Dilution Factor	Expected Value (pg/mL)	Measured Value (pg/mL)	Percent Recovery (%)
Serum A	none		657	
	7.5/10	493	489	99.3
	5.0/10	329	313	95.2
	2.5/10	164	147	89.5
Serum B	none		955	
	7.5/10	716	789	110.1
	5.0/10	478	525	109.9
	2.5/10	239	247	103.3
Serum C	none		1690	
	7.5/10	1268	1249	98.5
	5.0/10	845	884	104.6
	2.5/10	423	429	101.6

1c) Comparative Analysis (serum):

97 patient samples were analyzed with the AIA-PACK B12 assay and another commercially available vitamin B12 assay. The statistics, presented below, demonstrate good correlation between these two methods.

Slope	1.08
y-intercept	37.4 pg/mL
Correlation Coefficient	0.934
Range of Samples	271 - 1996 pg/mL
Number of Samples (n)	97

2) Precision

2a) Intra-assay precision

The intra-assay (within run) precision coefficient of variation was evaluated in four control samples by 10 replicate determinations.

Sample	Number of Replicates	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
Control A	10	101	18.6	18.4
Control B	10	349	32.0	9.1
Control C	10	775	54.0	7.0
Control D	10	1210	75.8	6.3

2b) Inter-assay precision

The inter-assay (between run) precision coefficient of variation was evaluated at four different concentrations by analyzing controls in triplicate in 20 to 23 separate runs.

Sample	Number of Replicates	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
Control A	23	109	7.5	6.9
Control B	20	343	9.8	2.9
Control C	20	701	33.6	4.8
Control D	20	1311	59.9	4.6

Specificity

The following substances were tested for cross-reactivity. The cross-reactivity (%) is the percent of the compound that will be identified as vitamin B12. If these compounds are present in the specimen at the same concentration as vitamin B12, the final result will be increased by these percentages.

Compound	Concentration	Cross-reactivity (%)
Cobinamide	200 ng/mL	<1.0

Sensitivity

The minimal detectable concentration (MDC) of vitamin B12 is estimated to be 50 pg/mL. The MDC is defined as that concentration of B12 which corresponds to the rate of fluorescence that is two standard deviations from the mean rate of fluorescence of 20 replicate determinations of a zero calibrator.

Interference

Interference is defined, for purposes of this study, to be recovery outside of 10% of the known specimen mean concentration.

- Added hemoglobin (up to 490 mg/dL), conjugated bilirubin (up to 14.3 mg/dL) and free bilirubin (up to 17.9 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 1667 mg/dL), does not interfere with the assay.
- Protein, as indicated by added albumin (up to 2.5 g/dL), does not interfere with the assay.
- Ascorbic acid (up to 20.0 mg/dL) does not interfere with the assay

References

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8) Tietz, N. W., ed, 1990, Clinical Guide to Laboratory Tests 2nd Ed, W.B. Saunders, p. 580-581.

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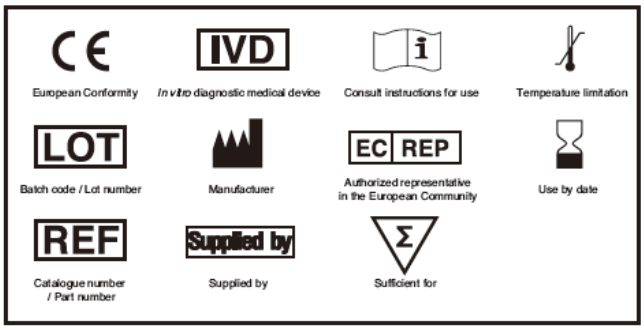


AIA-PACK
B12 Calibrator Set

Intended Use

The AIA-PACK B12 Calibrator Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration the AIA-PACK B12 Assay.

Summary and Explanation

The AIA-PACK B12 Calibrator Set contains buffered human serum albumin with assigned levels of cyanocobalamin (B12). Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators in this set are prepared gravimetrically and are compared to internal reference standards.

Material Provided (Cat. No. 020393)

2 x 1 mL	Calibrator	No. 1	0	pg/mL

Buffered human serum albumin containing no detectable concentration of vitamin B12 (0 pg B12/mL), and 0.1% sodium azide as a preservative.

2 x 1 mL	Calibrator	No. 2	100	pg/mL (approx.)
		No. 3	250	pg/mL (approx.)
		No. 4	500	pg/mL (approx.)
		No. 5	1000	pg/mL (approx.)
		No. 6	2100	pg/mL (approx.)

Buffered human serum albumin containing the assigned concentration of vitamin B12 (described on each vial), and 0.1% sodium azide as a preservative. (Lyophilized)

Warnings and Precautions

- The AIA-PACK B12 Calibrator Set is for in vitro diagnostic use.
- Calibrator (1) contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.
- The AIA-PACK B12 Pretreatment Set used in conjunction with the AIA-PACK B12 Calibrator Set contains 0.06% potassium cyanide and 1.6% sodium hydroxide. Avoid contact with eyes, skin or clothing. Wash thoroughly after handling. If discarded into drain, flush with a large volume of water.

Preparation and Storage

- Using volumetric pipettes, reconstitute the lyophilized calibrators accurately to the volume of 1 mL with CAP Class I or NCCLS (CLSI) Type I Reagent Grade water. Allow the lyophilized material to fully dissolve, then mix the calibrators gently but thoroughly prior to performing the calibration.
- Bring calibrator to room temperature (18° - 25° C) for use.
- Always store the Calibrator Set in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK B12 Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 24 hours. Upon completion of the pretreatment procedure, the calibrators should be assayed within 1 hour.

Calibration

Pretreatment

All calibrators must be pretreated prior to assay on the AIA Systems. On the AIA 600 II, AIA-900, AIA-1800 and AIA-2000, the pretreatment is performed automatically using the parameters defined in Assay Specifications. If manual pretreatment is desired, follow the instructions for the Manual Procedure.

Automatic Pretreatment Procedure

1. Bring all pretreatment reagents and samples to room temperature.
2. Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to the volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve.
3. Place the reconstituted Pretreatment-1 (PT-1) and Pretreatment-3 (PT-3) in the designated positions on the reagent cassette as defined under REAGENT REGISTRATION. Note: On the AIA-600 II, Pretreatment-3 is placed in the position designated as PRETREATMENT (2).
4. Place the appropriate amount of sample cups, sample treatment cups and AIA-PACK B12 test cups on the instrument.

Manual Pretreatment Procedure

1. Set all pretreatment parameters in the software to 0 (zero).
2. Bring all pretreatment reagents and calibrators to room temperature.
3. Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to the volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve.
4. Pipette 200 µL of calibrator into a glass or polypropylene tube.
5. Add 100 µL of the reconstituted Pretreatment-1 to each tube and mix well using a vortex mixer.
6. Incubate the treated calibrators for 15 minutes at room temperature in the dark.
7. Add 100 µL of the Pretreatment-3 to each treated calibrator. Mix well using a vortex mixer.
8. Calibrators should be assayed within 1 hour.

Calibration Procedure

Refer to the CALIBRATION PROCEDURE in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the AIA System Operator's Manual.

1) Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
2) Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup).
3) Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4) Tosoh recommends that all calibrators be run in triplicate.

Assignment of Values

The AIA-PACK B12 Calibrator Set contains assigned concentrations of vitamin B12. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0 to 2100 pg/mL of vitamin B12. The Calibrator Set has been prepared gravimetrically and is compared to internal reference standards.

Results

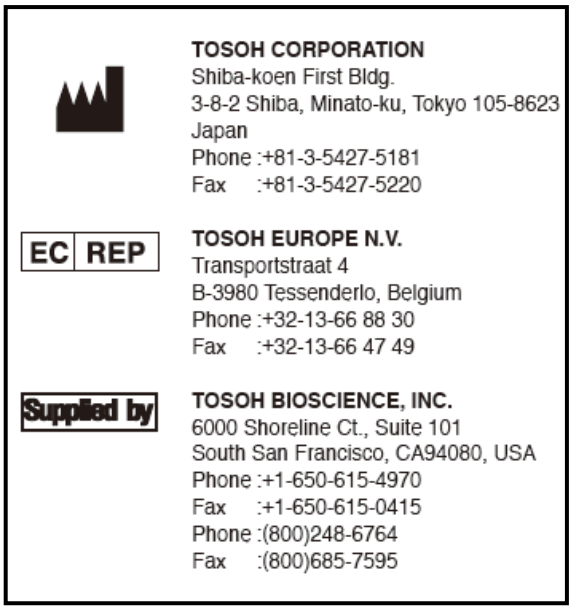
- Since there is an inverse relationship between concentration and rate, the rates should decrease as the concentration increases.
- The replicate values should be within a 10% range.

Limitations

The AIA-PACK B12 Calibrator Set is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Analyte Application Manual. Tosoh Bioscience, Inc., South San Francisco, CA.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., South San Francisco, CA.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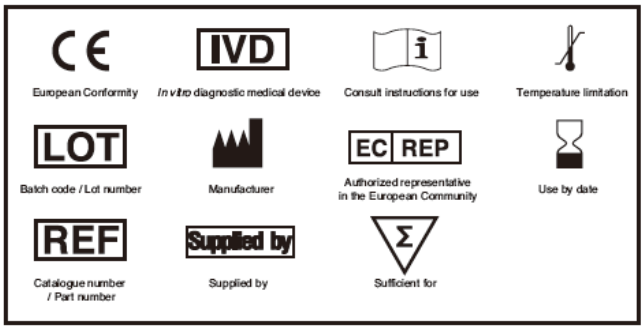
European Conformity
IVD
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
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Sufficient for

AIA-PACK
B12 Sample Diluting Solution

Intended Use

The AIA-PACK B12 Sample Diluting Solution is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples that have concentrations of vitamin B12 above the linear range of the assay.

Summary and Explanation

The AIA-PACK B12 Sample Diluting Solution contains human serum albumin with no detectable concentration of vitamin B12 (B12). This Sample Diluting Solution is to be used only with samples that are being tested for vitamin B12 concentrations using the AIA-PACK B12 assay.

Materials Provided (Cat. No. 020593)

4 x 4 mL	Sample Diluting Solution Human serum containing no detectable concentration of vitamin B12 (0 pg B12/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK B12 Sample Diluting Solution is for in vitro diagnostic use.
- These materials contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Human sera used in the preparation of this product has been tested by FDA cleared methods and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

- The AIA-PACK B12 Sample Diluting Solution is provided ready to use.
- Always store the Sample Diluting Solution in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK B12 Sample Diluting Solution is stable until the expiration date on the label. After opening, the Sample Diluting Solution is stable for up to 7 days when refrigerated at 2° - 8° C.

Procedure

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. If a specimen is found to contain greater than the linearity limit of approximately 2000 pg/mL, the specimen should be diluted with the Sample Diluting Solution and assayed according to the procedure in the AIA-PACK section of the analyte application.
2. The AIA-system will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for serum containing greater than 2000 pg/mL is 1:10. However, it is desirable to dilute the serum samples so that the diluted sample reads between 250 and 1500 pg B12/mL.

Results

When an auto-dilution is performed, the AIA instrument will calculate the final result.

Limitations

The AIA-PACK B12 Sample Diluting Solution is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Analyte Application Manual. Tosoh Bioscience, Inc., South San Francisco, CA.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., South San Francisco, CA.


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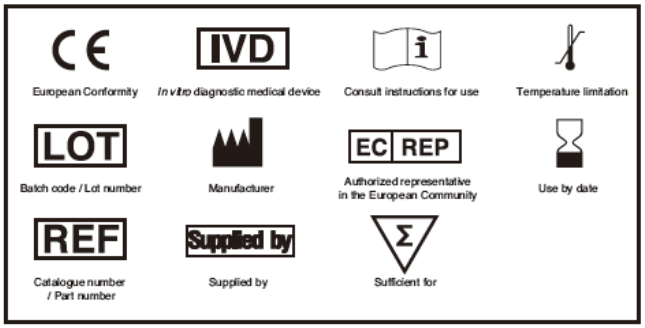
IVD
European Conformity
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
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AIA-PACK
B12 Pretreatment Set

Intended Use

The AIA-PACK B12 Pretreatment Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the pretreatment of serum specimens, calibrators and control material for the AIA-PACK B12 Assay.

Summary and Explanation

The AIA-PACK B12 Pretreatment Set is designed to convert the vitamin B12 (B12) in samples (human serum, calibrators, controls) to the measurable form of this analyte. The pretreatment should be performed according to the protocol described in the Pretreatment Procedure section of this insert.

Materials Provided (Cat. No. 020706)

6 vials	PRETREATMENT-1 Lyophilized. After reconstitution with 5 mL of PRETREATMENT-2, it will contain 1 mg/mL dithiothreitol (DTT) with 0.1% sodium azide as a preservative.
1 x 32 mL	PRETREATMENT-2 Aqueous solution containing 1.6% sodium hydroxide (NaOH) and 0.06% potassium cyanide (KCN).
6 x 5 mL	PRETREATMENT-3 Buffer solution with 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK B12 Pretreatment Set is for in vitro diagnostic use.
- The PRETREATMENT-1 and PRETREATMENT-3 contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such materials, always flush with large volumes of water to prevent azide build up.
- The PRETREATMENT-2 and the reconstituted PRETREATMENT-1 with the PRETREATMENT-2 contain 0.06% KCN and 1.6% NaOH. Avoid contact with eyes, skin or clothing. Wash thoroughly after handling. If discarded into drain, flush with a large volume of water.
- Pretreatment Sets with different lot numbers should not be combined.
- Pretreated patient samples should be handled with the same precautions as used for the samples before pretreatment.
- Do not use beyond the expiration date.

Preparation and Storage

- Using volumetric pipettes, reconstitute the lyophilized PRETREATMENT-1 accurately to the volume of 5 mL with the PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
- The PRETREATMENT-3 is provided ready to use.
- Always store the Pretreatment Set in an upright position at 2° - 8° C when not in use.

Stability

When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK B12 Pretreatment Set is stable until the expiration date on the label. Reconstituted PRETREATMENT-1 should be used within 24 hours.

Procedure

All samples (human serum, calibrators, and controls) must be pretreated prior to assay on the AIA Systems. On the AIA-600II, AIA-900, AIA-1800 and AIA-2000, the pretreatment is performed automatically using the parameters defined in Assay Specifications. If manual pretreatment is desired, follow the instructions for Manual Procedure. Refer to the AIA System Operator's Manual for additional instructions regarding assay procedure.

Automatic Pretreatment Procedure

1. Bring all pretreatment reagents and samples to room temperature.
2. Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to the volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve.
3. Place the reconstituted Pretreatment-1 (PT-1) and Pretreatment-3 (PT-3) in the designated positions on the reagent cassette as defined under REAGENT REGISTRATION. Note: Pretreatment-3 is placed in the position designated as PRETREATMENT (2).
4. Place the appropriate amount of sample cups/primary tubes, sample treatment cups and AIA-PACK B12 test cups on the instrument.

Manual Pretreatment Procedure

1. Set all pretreatment parameters in the software to 0 (zero).
2. Bring all pretreatment reagents and samples to room temperature.
3. Using a volumetric pipette, reconstitute the lyophilized Pretreatment-1 accurately to the volume of 5 mL with the Pretreatment-2. Allow the lyophilized material to fully dissolve.
4. Pipette 200 µL of sample (serum, control or calibrator) into a glass or polypropylene tube.
5. Add 100 µL of the reconstituted Pretreatment-1 to each tube and mix well using a vortex mixer.
6. Incubate the treated sample for 15 minutes at room temperature in the dark.
7. Add 100 µL of the Pretreatment-3 to each treated sample. Mix well using a vortex mixer.
8. Samples should be assayed within 1 hour.

Limitations

The AIA-PACK B12 Pretreatment Set is designed solely for use with AIA-PACK assay procedures.

References

1. AIA Analyte Application Manual. Tosoh Bioscience, Inc., South San Francisco, CA.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., South San Francisco, CA.

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