AIA-360 Assay Specifications
ST BMG Test Code 065

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0.000 mg/L
3	Cal 2	0.015 mg/L (example)
4	Cal 3	0.050 mg/L (example)
5	Cal 4	0.150 mg/L (example)
6	Cal 5	0.300 mg/L (example)
7	Cal 6	0.400 mg/L (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	mg/L
11	Smpl. Vol	15
12	Dil. Vol	135
13	Assay L	0.002
14	Assay H	0.4
15	Ref. L	
16	Ref. H	
17	Decimal	4
	Visible only in Test Mode	
18	Test Name	#BMG
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	4
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	1.000000
25	Factor1 B	0.000000
26	Factor2 A	1.000000
27	Factor2 B	0.000000
28	V. Conc	0.000000
29	G Origin	0.000000

AIA-900 Assay Specifications
ST BMG Test Code 065

No.	Item	Data
1	Code	65
2	ACT	1
3	Analyte	#BMG
4	Lot	***Enter current cal. lot no.
5	CAL 1	0.000 mg/L
6	CAL 2	0.015 mg/L (example)
7	CAL 3	0.050 mg/L (example)
8	CAL 4	0.150 mg/L (example)
9	CAL 5	0.300 mg/L (example)
10	CAL 6	0.400 mg/L (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	mg/L
14	Decimal	4
15	Assay Low	0.002
16	Assay High	0.4
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.102
20	Reschedule High	20.4
21	Factor A	1
22	Factor B	0
23	Sample Volume	15
24	Diluent Volume	135
25	2 Step reagent dispensing volume	0
26	Calibration code	4
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	4
30	CAL. CV	90
31	DIL. SP1	51
32	DIL. SP2	5
33	DIL. CAL. (Calculation of dil ratio of conc.)	1
34	DIL. CNTL.	51
35	DIL. DO	10
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	4
39	DIL. NAME	BMG
40	DIL. PRTY	3
41	PRE. SPVOL (Vol. of pretreated sample)	0
42	PRE. 1VOL (Vol. of pretreatment sol-1)	0
43	PRE. 2 VOL (Vol. of pretreatment sol-2)	0
44	PRE. CODE (pretreated sol. code)	0
45	PRE. NAME (pretreated sol. name)	0
46	Protocol	1
47	SYS. F_A	1
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications
ST BMG Test Code 065

No.	Item	Data
1	Unit	mg/L
2	Decimal places	4
3	Reference low	
4	Reference high	
5	Reschedule low	0.102
6	Reschedule high	20.4
7	Assay range low	0.0020
8	Assay range high	0.4
9	Specimen diluent code	4
10	Specimen diluent name	BMG
11	Dilution factor for Sp. 1	51
12	Dilution factor for Sp. 2	5
13	Dilution factor for Control	51
14	Default multiplier for DO	10
15	Default multiplier for >H	5
16	Factor A	1.00000e+000
17	Factor B	0.00000e+000
18	Calibration code	4
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0.000 mg/L
23	Cal - 02	0.015 mg/L (example)
24	Cal - 03	0.050 mg/L (example)
25	Cal - 04	0.150 mg/L (example)
26	Cal - 05	0.300 mg/L (example)
27	Cal - 06	0.400 mg/L (example)
28		
29		
30		
31		
32		
33		
34	Dilution factor for calibrator	1
35	Calibration period	90
36	Incubation time (10/40)	10
37	Assay protocol	1
38	Specimen volume	15
39	Diluent volume	135
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	0
43	Pretreatment specimen volume	0
44	Pretreatment Reagent code	0
45	Pretreatment Reagent name	0
46	Pretreatment Reagent 1 volume	0
47	Pretreatment Reagent 2 volume	0
48	System Factor	1
49	Calibration Code Check	004
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

β2 Microglobulin
ST AIA-PACK BMG

Name and Intended Use

ST AIA-PACK BMG is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of β2 Microglobulin (BMG) in human serum, plasma and urine on Tosoh AIA System analyzers.

Summary and Explanation of Test

Human β2 microglobulin (BMG) is a single polypeptide of 100 amino acids, identified in 1968 by Berggard et al.[1] The molecular weight of BMG is 11,800 daltons and represents the light-chain moiety of the major histocompatibility complex class antigens.[2-4] It is worth noting that BMG shows approximately 35% sequence homology with immunoglobulin domains. BMG is shed from the cell surface of all nucleated cells[2,3] during growth and differentiation, and cleared in the kidneys by filtration through the glomeruli. Since it is reabsorbed by the proximal tubular cells[5] and degraded, only trace amounts are detected in urine from healthy individuals, whereas marked elevation is seen in patients with proximal tubular dysfunctions due to exposure to aminoglycosides,[6] anti-cancer or inflammatory drugs[7,8] and heavy metals.[9]

Determination of serum BMG is of diagnostic value in a variety of disorders. It can be used to assess glomerular function in advanced renal disease[10,11] and after renal transplantation.[12,13] It can provide a measure of tumor burden and prognosis in patients with multiple myeloma,[14,15] B cell lymphoma[16] and chronic lymphocytic leukemia.[17] It is useful to monitor disease activity in chronic inflammatory disorders, such as rheumatoid arthritis,[18] systemic lupus erythematosis,[19] Sjogren's syndrome,[20] and Crohn's disease.[21] More recently, BMG has been reported to be of value in monitoring patients with HIV infection.[22-25]

Principle of the Assay

The ST AIA-PACK BMG is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. β2 microglobulin present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled polyclonal antibody in the AIA-PACK. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the BMG concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK BMG, Cat. No. 0025259)

5 trays x 20 test cups (ST AIA-PACK BMG Test Cup)

Plastic test cups containing lyophilized magnetic beads coated with anti-β2 microglobulin mouse monoclonal antibody and anti-β2 microglobulin rabbit polyclonal antibody 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 β2 microglobulin analysis using the ST AIA-PACK BMG (Cat. No. 0025259) on specific Tosoh AIA Systems. They are available separately from Tosoh.

Materials	Cat. No.
AIA-SYSTEMS:	
AIA-360	0019945
AIA-900	0022930
AIA-900 9tray Sorter	0022931
AIA-900 19tray Sorter	0022932
AIA-2000 ST	0022100
AIA-2000 LA	0022101
AIA-PACK:	
AIA-PACK Substrate Set II	0020968
AIA-PACK Substrate/Reconstituent	
AIA-PACK BMG Calibrator Set	0020359
Calibrator #1 0.000 mg/L	
Calibrator #2 0.015 mg/L (approx.)	
Calibrator #3 0.050 mg/L (approx.)	
Calibrator #4 0.150 mg/L (approx.)	
Calibrator #5 0.300 mg/L (approx.)	
Calibrator #6 0.400 mg/L (approx.)	
AIA-PACK BMG Sample Diluting Solution	0020559
AIA-PACK Wash Concentrate Set	0020955
AIA-PACK Diluent Concentrate Set	0020956
AIA-PACK Detector Standardization Test Cups	0020970
AIA-PACK Sample Treatment Cups	0020971
Sample Cups	0018581
ADDITIONAL REQUIREMENTS: (Except AIA-360)	
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 ST AIA-PACK BMG is intended for in vitro diagnostic use only.
- ℞ only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- Test cups from different lots or different assays shall not be mixed within a tray.
- The ST AIA-PACK BMG 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.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- Do not use beyond the expiration date.
- The ST AIA-PACK BMG has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. On the recommended 51 fold dilution, serum or plasma samples with BMG concentrations between 20.4 and 102 mg/L will read > 0.400 mg/L. On the recommended 5 fold dilution for urine, samples with BMG concentrations between 2.0 and 10.0 mg/L will read > 0.400 mg/L. Using the recommended initial dilutions, the "hook effect" phenomenon may occur at BMG serum or plasma concentrations >102 mg/L and urine concentrations > 10.0 mg/L.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies, should not be used with samples from patients under Asfotase Alfa treatment.

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):	
ST AIA-PACK BMG	0025259
AIA-PACK BMG Calibrator Set	0020359
AIA-PACK BMG Sample Diluting Concentrate	0020559
AIA-PACK Substrate Set II	0020968
AIA-PACK Wash Concentrate	0020955

AIA-PACK Diluent Concentrate	0020956
Room Temperature (18 - 25 °C):	
Detector Standardization Test Cups	0020970
Sample Treatment Cups	0020971

ST AIA-PACK BMG 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. After reconstituting, Sample Diluting Solution is stable for up to 90 days refrigerated at 2 - 8 °C. Reconstituted substrate solution is stable for 3 days at 18 - 25 °C or 30 days at 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

Either serum, heparinized plasma or urine may be used for this assay. EDTA and citrated plasma SHOULD NOT BE USED.

No special patient preparation is necessary. When using serum, a venous blood sample is collected aseptically without additives. Store at 18 - 25 °C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain the serum specimen for assay.

To use heparinized plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.

Urine should be collected in a container to which no preservatives have been added. After collection the urine pH should be adjusted to read 7-8 if the testing will not be done immediately.

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 dilution prior to analysis. Serum and plasma samples are diluted 1:51 and urine samples 1:5. In the AIA-900 and AIA-2000 dilution factors are identified in the test file as Sp.1 for serum (1:51) and Sp.2 for urine (1:5).

The sample volume required for analysis is 10 µL of serum or plasma and 50 µL of urine.

Procedure

1. Sample Preparation

For manual dilutions:

Serum or plasma samples should be diluted 1:51 with AIA-PACK BMG Sample Diluting Solution (1 part sample + 50 parts Sample Diluting Solution).

Urine samples should be diluted 1:5 with AIA-PACK BMG Sample Diluting Solution (1 part sample + 4 parts Sample Diluting Solution).

Control material should be prepared according to manufacturer's instructions.

2. Reagent Preparation

2a) 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.

2b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L.

2c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

2d) Sample Diluting solution

Add 90 mL of CAP Class I water or the clinical laboratory reagent water to the BMG Sample Diluting Concentrate (10mL) and mix well.

3. Calibration

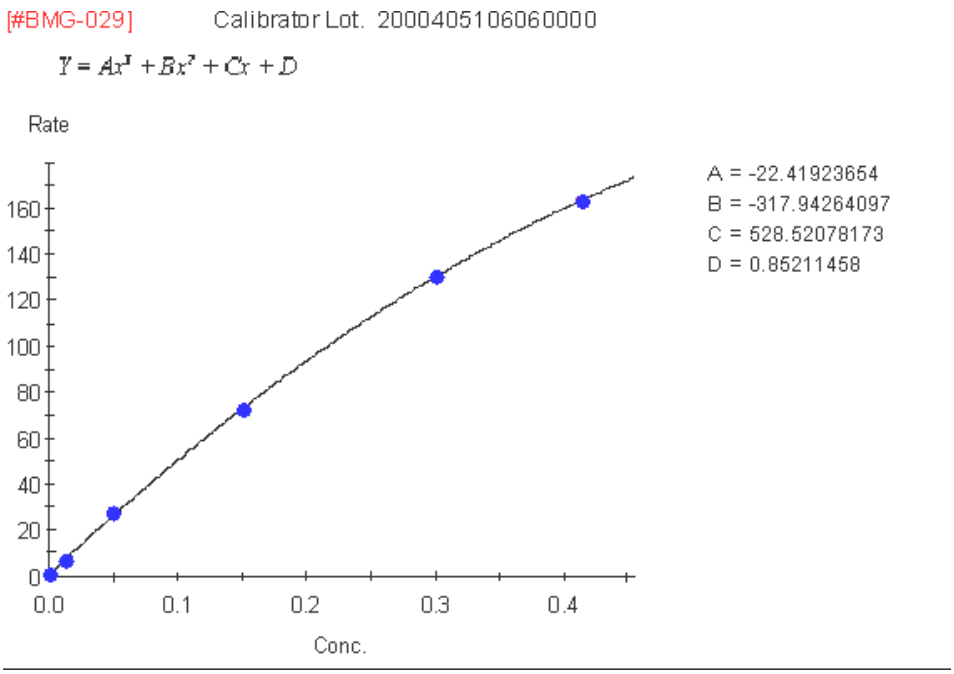
3a) Calibration Curve

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

The calibration curve for the ST AIA-PACK BMG is stable for up to 90 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). 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.



3a) Calibration Procedure

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

1) Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
2) The AIA-PACK BMG CALIBRATOR (1) is provided ready for use.
3) Calibrator levels (2) - (6) for ST AIA-PACK BMG are lyophilized. Lyophilized calibrators should be reconstituted with 1.0 mL of CAP Class I water or the clinical laboratory reagent water.
4) Tosoh recommends that all calibrators be run in triplicate.

3b) Calibration Acceptability criteria

1) The mean rate for the zero calibrator should be < 3.0 nM/sec.
2) Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
3) The replicate values should be within a 10% range.

3c) Calibration Review and Acceptance

1) Review the calibration curve carefully, using the criteria listed above.
2) Edit the calibration if necessary, then accept the calibration.
3) 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

1. 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.
2. 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 the analyzer, place samples on the instrument appropriately.

5b) Assay Procedure

1. Ensure a sufficient quantity of ST AIA-PACK BMG test cups for the number of samples to be run.
2. Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA-900 and AIA-2000 will require AIA-PACK Sample Treatment Cups if onboard dilutions are utilized.

Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water. 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 CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.
3. If a specimen β2 microglobulin concentration is found to contain greater than the linearity limit of the assay, 0.4 mg/L (20.4 after x51 dilution factor), the specimen should be diluted further with the BMG Sample Diluting Solution and re-assayed according to the assay procedure.

The recommended further dilution for samples is 1:10. The re-diluted serum sample should read between 2.5 - 15 mg/L. Urine samples should be diluted so that the further dilution reads between 0.25 - 1.5 mg/L. If the autodilution feature is not being used, the further dilution must be made using the original 1:51 or 1:5 dilution.

4. The AIA systems can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK BMG Test cups can be used during the same run.
5. If the assay specifications for this test are not already in the system software, the specifications must be entered under test code 065.
6. The AIA-PACK BMG has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. On the recommended 51 fold dilution, serum samples with BMG concentrations between 20.4 and 102 mg/L will read > 0.400 mg/L. On the recommended 5 fold dilution for urine, samples with BMG concentration between 2.0 and 10.0 mg/L will read > 0.400 mg/L. Using the recommended initial dilutions, the "hook effect" phenomenon may occur at BMG serum concentrations > 102 mg/L, and urine concentrations > 10.0 mg/L.

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 β2 microglobulin concentration in mg/L.

For detailed information regarding programming dilutions, consult the appropriate Operator's Manual.

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, two levels of controls are run in order to accept the calibration curve.
- The two 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 ST AIA-PACK BMG, all samples must be prediluted with BMG Sample Diluting Solution. Using the recommended dilutions, the assay linearity of approximately 0.4 mg/L equates to calculated values of 20.4 mg/L in serum and 2.0 mg/L in urine. The lowest measurable concentration of BMG is 0.002 mg/L (raw value), which equates to 0.102 mg/L in serum, and 0.010 mg/L in urine. Specimens that read < 0.002 mg/L may be repeated on a lesser dilution, and results calculated using the correct dilution factor.

The exact linearity of the ST AIA-PACK BMG depends on the particular lot of calibrator in use. Although the approximate value of the highest calibrator is 0.4 mg/L, the exact concentration may be slightly different. The assay specification, Assay Range High, should be defined as the upper limit of the assay range, 0.4 mg/L.

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.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated values when tested for β2 microglobulin.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. 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.[26]

Reference Ranges

The intervals given here were determined in 92 serum samples and 54 urine samples from apparently healthy individuals.

Category	Value
Serum	< 2.7 mg/L
Urine	< 0.3 mg/L

Performance Characteristics

The following performance characteristics were established using the Tosoh AIA NexIA. All the systems demonstrate equivalent performance.

1) Accuracy

1a) Recovery:

Three serum pools and three urine pools were spiked with three different levels of β2 microglobulin and assayed before and after spiking.

Sample	Initial Value (mg/L)	BMG Added (mg/L)	Expected Value (mg/L)	Measured Value (mg/L)	Percent Recovery (%)
Serum A1	0.021	0.094	0.115	0.116	100.9
	0.021	0.047	0.068	0.065	95.6
	0.021	0.023	0.044	0.042	95.5
Serum B1	0.021	0.094	0.115	0.120	104.3
	0.021	0.047	0.068	0.068	100.0
	0.021	0.023	0.044	0.044	100.0
Serum C1	0.026	0.094	0.120	0.127	105.8
	0.026	0.047	0.073	0.072	98.6
	0.026	0.023	0.049	0.050	102.0
Urine A1	0.021	0.248	0.269	0.256	95.2
	0.021	0.124	0.145	0.138	95.2
	0.021	0.062	0.083	0.076	91.6
Urine B1	0.000	0.248	0.248	0.240	96.8
	0.000	0.124	0.124	0.110	88.7
	0.000	0.062	0.062	0.054	87.1
Urine C1	0.015	0.248	0.263	0.250	95.0
	0.015	0.124	0.139	0.129	92.8
	0.015	0.062	0.077	0.069	89.6

1b) Dilution:

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

Sample	Dilution Factor	Expected Value (mg/L)	Measured Value (mg/L)	Percent Recovery (%)
Serum A2	none		0.397	
	7.5/10	0.298	0.285	95.6
	5.0/10	0.199	0.188	94.5
	2.5/10	0.099	0.091	91.9
	1.0/10	0.040	0.036	90.0
Serum B2	none		0.352	
	7.5/10	0.264	0.256	97.0
	5.0/10	0.176	0.162	92.0
	2.5/10	0.088	0.079	89.8
	1.0/10	0.035	0.031	88.6
Serum C2	none		0.340	
	7.5/10	0.255	0.261	102.3
	5.0/10	0.170	0.178	104.7
	2.5/10	0.085	0.087	102.4
	1.0/10	0.034	0.036	105.9
Urine A2	none		0.377	
	7.5/10	0.283	0.272	96.1
	5.0/10	0.189	0.185	97.9
	2.5/10	0.094	0.086	91.5
	1.0/10	0.038	0.035	92.1
Urine B2	none		0.383	
	7.5/10	0.287	0.269	93.7
	5.0/10	0.191	0.182	95.3
	2.5/10	0.096	0.086	89.6
	1.0/10	0.038	0.035	92.1
Urine C2	none		0.413	
	7.5/10	0.310	0.303	97.8
	5.0/10	0.207	0.205	99.0
	2.5/10	0.103	0.097	94.2
	1.0/10	0.041	0.038	92.7

2) Precision

2a) Intra-assay precision

The intra-assay (within run) precision was determined using five controls in a total of 20 runs. Within each run, one set of duplicates per control was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation.

Sample	Mean (mg/L)	Pooled SD (mg/L)	Coefficient of Variation (%)
Serum A3	0.023	0.00074	3.2
Serum B3	0.103	0.00250	2.4
Serum C3	0.247	0.00504	2.0
Urine A3	0.028	0.00047	1.7
Urine B3	0.227	0.00397	1.7

2b) Inter-assay precision

The inter-assay (between run) precision coefficient of variation was evaluated at five different concentrations, three in serum and two in urine, by analyzing samples in 20 separate runs over 20 days.

Sample	Number of Replicates	Mean (mg/L)	Standard Deviation (mg/L)	Coefficient of Variation (%)
Serum A3	20	0.023	0.00108	4.7
Serum B3	20	0.102	0.00322	3.2
Serum C3	20	0.243	0.00809	3.3
Urine A3	20	0.028	0.00085	3.1
Urine B3	20	0.225	0.00566	2.5

2c) Total precision

Total precision was determined by the duplicate assay of five controls in 20 separate runs. The means of each run were used to calculate the standard deviation (SD) and coefficient of variation (CV).

Sample	Mean (mg/L)	Standard Deviation (mg/L)	Coefficient of Variation (%)
Serum A3	0.023	0.00109	4.7
Serum B3	0.103	0.00386	3.7
Serum C3	0.247	0.00932	3.8
Urine A3	0.028	0.00087	3.1
Urine B3	0.227	0.00745	3.3

Sensitivity

The minimal detectable concentration of β2 microglobulin is estimated to be 0.002 mg/L as the "instrument measured concentration". For a 1:5 dilution of urine, this equates to 0.01 mg/L; for a 1:51 dilution of serum, this equates to a concentration of 0.10 mg/L. The minimal detectable concentration is defined as that concentration of β2 microglobulin 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 390 mg/dL), free bilirubin (up to 17 mg/dL) and conjugated bilirubin (up to 19 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by triglyceride concentrations (up to 1,660 mg/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
- Protein, as indicated by human albumin concentrations (up to 5 g/dL), does not interfere with the assay.
- Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies, should not be used with samples from patients under Asfotase Alfa treatment.

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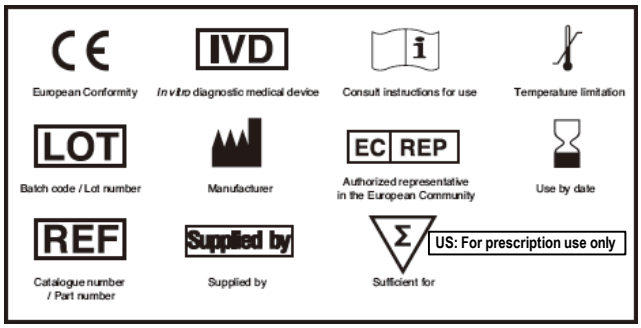

CE
European Conformity
IVD
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
Supplied by
Σ
Sufficient for
US: For prescription use only

AIA-PACK
BMG Calibrator Set

Intended Use

The AIA-PACK BMG Calibrator Set is intended for IN VITRO diagnostic use only for the calibration of the ST AIA-PACK BMG assays.

Summary and Explanation

The AIA-PACK BMG Calibrator Set contains buffered bovine serum albumin with assigned levels of β2 microglobulin. 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 compared to internal reference standards.

Material Provided (Cat. No. 0020359)

2 x 1 mL	Calibrator	No. 1	0.0	mg/L

Buffered bovine serum albumin containing no detectable concentration of BMG (0 mg BMG/L), and 0.1% sodium azide as preservative (ready for use).

2 x 1 mL	Calibrator	No. 2	0.015	mg/L	(approx.)
		No. 3	0.050	mg/L	(approx.)
		No. 4	0.150	mg/L	(approx.)
		No. 5	0.300	mg/L	(approx.)
		No. 6	0.400	mg/L	(approx.)

Buffered bovine serum albumin containing the assigned concentration of BMG (described on each vial) and 0.1% sodium azide as preservative (lyophilized).

Warnings and Precautions

- The AIA-PACK BMG Calibrator Set 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.
- Although material derived from human blood is not used for BMG Calibrators, it is recommended that this product be handled with the same precautions used for patient samples.
- Do not use beyond the expiration date.

Preparation and Storage

Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized calibrators accurately to the volume of 1.0 mL with CAP Class I water or the clinical laboratory reagent 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.

ST BMG Calibrator materials do not require dilution prior to assay.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK BMG Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 24 hours.

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.

Calibration

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer 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 BMG Calibrator Set contains assigned concentrations of β2 microglobulin. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0.0 to 0.400 mg/L of β2 microglobulin. The calibrators in this set have been prepared gravimetrically and are compared to internal reference standards.

Results

The mean rate for the zero calibrator should be <3.0 nM/sec.

Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.

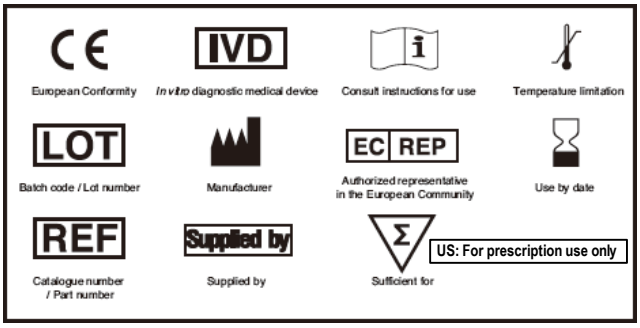
The replicate values should be within a 10% range.

Limitations

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

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.



AIA-PACK
BMG Sample Diluting Concentrate

Intended Use

The AIA-PACK BMG Sample Diluting Concentration is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute all patient serum, plasma and urine β2 microglobulin samples.

Summary and Explanation

The AIA-PACK BMG Sample Diluting Solution contains buffered bovine serum albumin with no detectable concentration of β2 microglobulin (BMG). This Sample Diluting Solution is to be used only with samples that are being tested for BMG concentrations using the ST AIA-PACK BMG assays.

Material Provided (Cat. No. 0020559)

4 x 10 mL Sample Diluting Concentrate

Buffered bovine serum albumin containing no detectable concentration of β2 microglobulin (0 mg BMG/L), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK BMG Sample Diluting Concentrate 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.
- Although material derived from human blood is not used for BMG Sample Diluting Solution, 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

- Add 90 mL of CAP Class I water or the clinical laboratory reagent water to the BMG Sample Diluting Concentrate (10ml) and mix well.
- 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 BMG Sample Diluting Concentrate is stable until the expiration date on the label. After reconstituting, the Sample Diluting Solution is stable for up to 90 days when refrigerated at 2 - 8 °C.

Procedure

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

1. Using AIA-PACK BMG or ST AIA-PACK BMG, all samples must be prediluted with BMG Sample Diluting Solution. Serum and plasma specimens are diluted 1:51 and urine specimens are diluted 1:5 prior to analysis. Using the recommended dilutions, the assay linearity of approximately 0.400 mg/L (raw value) equates to 20.4 mg/L in serum or plasma, and 2.0 mg/L in urine. The lowest measurable concentration of BMG is 0.002 mg/L (raw value), which equates to 0.102 mg/L in serum or plasma, and 0.010 mg/L in urine.
2. Diluted specimens that read < 0.002 mg/L may be repeated on a lesser dilution, and results calculated using the correct dilution factor.
3. If a diluted specimen is found to contain greater than the linearity limit of approximately 0.400 mg/L, the specimen should be further diluted with the Sample Diluting Solution and assayed according to the procedure in the AIA-PACK section of the analyte application.
4. The recommended further dilution for samples is 1:10. However, it is desirable to dilute the serum specimens so that the diluted sample reads between 0.25 - 1.5 mg/L (0.05 - 0.30 mg/L prior to calculating the result). Urine specimens containing more than 2.0 mg/L BMG should be diluted so that the further dilution reads between 0.25 - 1.5 mg/L (0.05 - 0.30 mg/L prior to calculating the result).
5. The AIA 900 and AIA-2000 will automatically perform the 1:51 or 1:5 dilution as defined in the Assay Specifications and calculate the result.
6. If the autodilution feature is not being used, the further dilution must be made using the original 1:51 or 1:5 dilution.

Results

If autodilution is used, the AIA instrument will automatically perform the 1:51 or 1:5 dilution as defined in the Assay Specifications and calculate the result.

Limitations

The AIA-PACK BMG Sample Diluting Concentrate is designed solely for use with AIA-PACK assay procedures.

The exact linearity of the BMG assay depends upon the particular lot of calibrator in use. Although the approximate highest calibrator value is 0.400 mg/L, the exact concentration may be slightly different. The assay specification, ASSAY RANGE HIGH, should be defined as the exact concentration of the highest calibrator.

References

1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual Tosoh Bioscience, Inc., Grove City, OH.