

AIA-360 Assay Specifications

ST CKMB Test Code 078

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/mL
3	Cal 2	10 ng/mL (example)
4	Cal 3	20 ng/mL (example)
5	Cal 4	100 ng/mL (example)
6	Cal 5	200 ng/mL (example)
7	Cal 6	500 ng/mL (example)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/mL
11	Smpl. Vol	50
12	Dil. Vol	100
13	Assay L	0.5
14	Assay H	500
15	Ref. L	
16	Ref. H	
17	Decimal	1

Visible only in Test Mode

18	Test Name	#CKMB
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	3
22	Calib. CV	90
23	Assay Prtl	1
24	Factor1 A	3.320000
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 CKMB Test Code 078

No.	Item	Data
1	Code	78
2	ACT	1
3	Analyte	#CKMB
4	Lot	***Enter current cal lot no.
5	CAL 1	0 ng/mL
6	CAL 2	10 ng/mL (example)
7	CAL 3	20 ng/mL (example)
8	CAL 4	100 ng/mL (example)
9	CAL 5	200 ng/mL (example)
10	CAL 6	500 ng/mL (example)
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/mL
14	Decimal	1
15	Assay Low	0.5
16	Assay High	500
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.5
20	Reschedule High	500
21	Factor A	1
22	Factor B	0
23	Sample Volume	50
24	Diluent Volume	100
25	2 Step reagent dispensing volume	0
26	Calibration code	95
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	3
30	CAL. CV	90
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	95
39	DIL. NAME	CKMB
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	3.32
48	SYS. F_B	0
49	V. CONC.	0
50	G. ORIGIN	0

AIA-2000 Assay Specifications

ST CKMB Test Code 078

No.	Item	Data
1	Unit	ng/mL
2	Decimal places	1
3	Reference low	
4	Reference high	
5	Reschedule low	0.5
6	Reschedule high	500
7	Assay range low	0.5
8	Assay range high	500
9	Specimen diluent code	95
10	Specimen diluent name	CKMB
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	3
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	10 ng/mL (example)
24	Cal - 03	20 ng/mL (example)
25	Cal - 04	100 ng/mL (example)
26	Cal - 05	200 ng/mL (example)
27	Cal - 06	500 ng/mL (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	50
39	Diluent volume	100
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	3.32
49	Calibration Code Check	095
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Creatine Kinase MB Isoenzyme ST AIA-PACK CK-MB

Name and Intended Use

ST AIA-PACK CK-MB is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Creatine Kinase MB Isoenzyme (CK-MB) in human serum and heparinized plasma on specific Tosoh AIA System analyzers.

Summary and Explanation of Test

Creatine kinase (CK) is an enzyme found in many human tissues, including skeletal muscle, myocardium, and the brain. The molecule is a dimer and exists as three major isoenzyme structures, composed of either two M monomers (CK-MM), two B monomers (CK-BB), or one M and one B monomer (CK-MB). These dimers differ in molecular structure as well as tissue of primary source. CK-MM is primarily found in skeletal muscle, CK-BB is found predominantly in the brain and intestinal tissue, while CK-MB is seen in greatest concentration in cardiac muscle.

All forms of CK function in the reversible phosphorylation of creatine.^{1,2} According to the literature, increased serum concentration of each isoenzyme is associated with a particular disease state.

Serum CK-MM is usually consistent with skeletal muscle disease (e.g. Duchenne's muscular dystrophy) or skeletal muscle trauma.

Serum CK-BB elevation has been related to multiple conditions including Reye's syndrome, malignant hyperthermia, acute brain injury, cardiac failure, neurosurgical procedures and can be found even in some types of carcinoma such as gastric or prostatic.

The MB isoenzyme is widely used in the diagnosis of acute myocardial infarction.³ CK activity in serum begins to increase 4-6 hours following myocardial infarction, with peak activity reached between 18 and 30 hours. CK-MB isoenzyme returns to a normal level very rapidly, often within 24-36 hours. The diagnosis of acute myocardial infarction (AMI) is supported when serial serum samples from suspected patients show this characteristic elevation and then decline of CK-MB over the specified period of time (usually 2-36 hours).

It is this temporal pattern rather than the abnormal elevation in CK-MB which is indicative of the AMI. CK-MB can also be released from damaged cardiac cells in necrosis or trauma.

Elevations of CK-MB have also been reported to be found in regenerative disorders.⁴

There are limitations to the use of an abnormal CK-MB as an indication of AMI, therefore, all pertinent information such as clinical history, ECG results, LD1 isoenzyme abnormalities, ratio of CK-MB : total CK and the pattern of changes in CK-MB levels in serial samples should be taken into account when making the diagnosis of AMI.

Many methods are available for the separation and quantification of CK isoenzymes, including electrophoresis, ion-exchange chromatography, immunoinhibition, competitive immunoassays and immunometric methods. These procedures may measure either the enzymatic activity of the CK-MB isoenzyme or the mass concentration of the CK-MB fraction. The ST AIA-PACK CK-MB is a highly specific mass assay which is not affected by high levels of the other CK isoenzymes.

Principle of the Assay

The ST AIA-PACK CK-MB is a two-site immunoenzymetric assay which is performed entirely in the AIA-PACK. CK-MB present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal 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 CK-MB concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Material Provided (ST AIA-PACK CK-MB, Cat. No. 0025269)

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

Plastic test cups containing lyophilized magnetic beads coated with anti-CK-MB mouse monoclonal antibody and mouse monoclonal antibody (to human CK-MB) 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 Creatine Kinase MB Isoenzyme analysis using the ST AIA-PACK CK-MB (Cat. No. 0025269) 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 CKMB Calibrator Set	0020369
Calibrator #1 0 ng/mL	
Calibrator #2 10 ng/mL (approx.)	
Calibrator #3 20 ng/mL (approx.)	
Calibrator #4 100 ng/mL (approx.)	
Calibrator #5 200 ng/mL (approx.)	
Calibrator #6 500 ng/mL (approx.)	
AIA-PACK CKMB Sample Diluting Solution	0020569
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 CK-MB is intended for in vitro diagnostic use only.
- **Rx** 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 CK-MB 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 CK-MB has been designed so that the high dose “hook effect” is not a problem for the vast majority of samples. Samples with CK-MB concentrations between 500 and 10,000 ng/mL will read > 500 ng/mL. The “hook effect” phenomenon may occur at CK-MB concentrations > 10,000 ng/mL.
- 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 CK-MB	0025269
AIA-PACK CKMB Calibrator Set	0020369

AIA-PACK CKMB Sample Diluting Solution	0020569
AIA-PACK Substrate Set II	0020968
AIA-PACK Wash Concentrate	0020955
AIA-PACK Diluent Concentrate	0020956
Room Temperature (18 - 25 °C):	
AIA-PACK Detector Standardization Test Cups	0020970
AIA-PACK Sample Treatment Cups	0020971

ST AIA-PACK CK-MB 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 opening, 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

Serum or heparinized plasma may be used for the assay. EDTA and citrated plasma **SHOULD NOT BE USED**.

No special patient preparation is necessary. However, blood should be collected serially every 6 - 8 hours in order to detect the typical rise and fall of the CK-MB which is characteristic of AMI. Collection less often may result in missing the CK-MB temporal pattern.

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.

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.

The sample required for analysis is 50 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 water or the clinical laboratory reagent 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 water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

2) Calibration

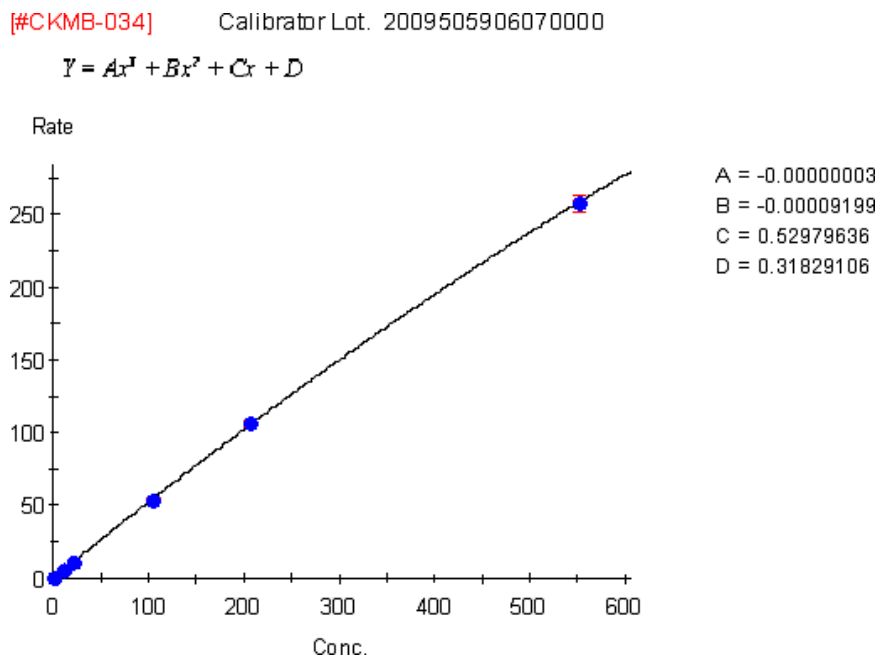
2a) Calibration Curve

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

The calibration curve for the ST AIA-PACK CK-MB 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.



2b) Calibration Procedure

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

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

2c) Calibration Acceptability criteria

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

2d) Calibration Review and Acceptance

- i) Review the calibration curve carefully, using the criteria listed above.
- ii) Edit the calibration if necessary, then accept the calibration.
- iii) For further information regarding calibration, consult the specific AIA System Operator's Manual.

3) Quality Control

3a) 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.

3b) Quality Control Procedure

- i) Assay quality control specimens as instructed in the specific Operator's Manual for the 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.

4) Specimen Processing

4a) Preparation

Following specific instructions in the Operator's Manual for the analyzer, place samples on the instrument in the appropriate positions.

4b) Assay Procedure

- i) Verify a sufficient quantity of ST AIA-PACK CK-MB 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-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 serum specimen Creatine Kinase MB Isoenzyme concentration is found to be greater than the linearity limit of the assay, 500ng/mL; the specimen should be diluted with the AIA-PACK CK-MB Sample Diluting Solution and re-assayed according to the Assay Procedure. The recommended dilution for samples containing greater than the concentration of the highest calibrator is 1:10 or 1:100. It is desirable to dilute the serum sample so that the diluted sample reads between 0.5 and 500 ng/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.
- 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 CK-MB 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 078.

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 Creatine Kinase MB concentration in ng/mL.

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 CK-MB, the highest concentration of Creatine Kinase MB Isoenzyme measurable without dilution is approximately 500 ng/mL, and the lowest measurable concentration is 0.5 ng/mL (assay sensitivity).

The exact linearity of the ST AIA-PACK CK-MB depends on the particular lot of calibrator in use. Although the approximate value of the highest calibrator is 500 ng/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, 500 ng/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.

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 CK-MB.

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. ¹¹

Reference Ranges

The interval given here was determined in serum samples from 103 non-hospitalized apparently healthy individuals.

Reference interval = < 5.8 ng/mL

Mean value = 1.3 ng/mL

95% of these samples gave results less than 2.8 ng/mL

As stated previously, there are cases in which a patient's serum, may contain elevated CK-MB concentrations unrelated to myocardial infarction. Such cases usually involve significant tissue trauma, and patients will exhibit increased levels of total CK activity. Because the activity of CK-MB in non-cardiac tissue is significantly less than the total CK activity in these tissues, a ratio of CK-MB to total CK may be calculated to further aid in the interpretation of the CK-MB result. The calculation of the CK-MB : CK ratio is analogous to the calculation of the % MB as obtained by electrophoresis. The expected range of the CK-MB : CK ratio is dependent on the reference (expected) range of total CK. Each laboratory should establish its own cutoff for the CK-MB : CK ratio (relative index).

It is recommended that different sample types not be mixed when performing repeat or serial testing on the same patient. A separate reference interval should be established for each sample type.

Performance Characteristics

1) Accuracy

1a) Recovery:

Three serum pools were spiked with three different levels of CK-MB and assayed before and after spiking.

Sample	Initial Value (ng/mL)	CK-MB Added (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A1	0.86	97.5	98.4	102.0	103.7
	0.86	195.0	195.9	205.5	104.9
	0.86	390.1	390.9	408.4	104.5
Serum B1	0.76	97.5	98.3	98.5	100.3
	0.76	195.0	195.8	210.9	107.7
	0.76	390.1	390.8	405.9	103.8
Serum C1	0.66	97.5	98.2	102.4	104.3
	0.66	195.0	195.7	201.5	103.0
	0.66	390.1	390.7	415.0	106.2

1b) Dilution:

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

Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A2	none		450.3	
	7.5/10	337.7	339.7	100.6
	5.0/10	225.2	218.5	97.0
	2.5/10	112.6	106.9	94.9
	1.0/10	45.0	41.4	91.9
Serum B2	none		491.7	
	7.5/10	368.8	370.0	100.3
	5.0/10	245.8	249.7	101.6
	2.5/10	122.9	123.9	100.8
	1.0/10	49.2	47.9	97.4
Serum C2	none		201.8	
	7.5/10	151.3	155.4	102.7
	5.0/10	100.9	103.2	102.3
	2.5/10	50.4	48.3	95.8
	1.0/10	20.2	18.6	92.4

2) Precision

2a) Intra-assay precision

The intra-assay (within run) precision was determined using three 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 (CV).

Sample	Mean (ng/mL)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Serum A3	9.94	0.380	3.8
Serum B3	97.7	2.38	2.4
Serum C3	287.6	7.25	2.5

2b) Inter-assay precision

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

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A	20	9.99	0.38	3.8
Serum B	20	96.92	2.92	3.0
Serum C	20	284.52	6.79	2.4

2c) Total precision

Total precision was determined by the duplicate assay of three 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 (ng/mL)	SD (ng/mL)	Coefficient of Variation (%)
Serum A3	9.94	0.366	3.7
Serum B3	97.7	2.55	2.6
Serum C3	287.6	8.46	2.9

Specificity

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

Compound	Cross-reactivity (%)
CK-MB	100
CK-MM	<0.0
CK-MB	<0.4

Sensitivity

The minimal detectable concentration (MDC) of Creatine Kinase MB Isoenzyme is estimated to be 0.5 ng/mL. The MDC is defined as that concentration of CK-MB 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 added 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/L), 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.

References

- 1) Lang, H.(ed)., 1981, Creatine Kinase Isoenzymes, Pathophysiology and Clinical Application. Springer-Verlang: New York.
- 2) Galen, R.S., Gambino, R., 1975, Isoenzymes of CPK and LDH in Myocardial Infarction and Certain Other Diseases. Pathobiol. Annul 5: 283-315.
- 3) McQueen, M., Holder, D., El-Maraghi, N., 1983, Assessment of the Accuracy of Serial Electrocardiograms in the Diagnosis of Myocardial Infarction. Amer. Heart J., 105: 258-261.
- 4) Navin, T.R., Hager, D.W., 1979, Creatine Kinase MB Isoenzyme in the Evaluation of Myocardial Infarction. Current Problems in Cardiology, Vol. III, No. 12, March.
- 5) Tietz, N.W.(ed)., 1976, Fundamentals of Clinical Chemistry. W.B. Saunders Company: Philadelphia.
- 6) Kaplan, L.A., Pesce, A.J.(ed).,1989, Clinical Chemistry Theory, Analysis, and Correlation. C.V. Mosby Company, St. Louis.
- 7) Kiyuse, J.Y, 1985, Current Status of detecting CK-MB for patient management, Am. Clin. Prod. 4: 29-31.
- 8) Bias, R., Edwards, J.B., 1982, Creatine Kinase (Review) Crit. Rev. Clin. Lab. Sci. 16: 291-335.
- 9) Fenton, J.J., et al, 1984, Diagnostic Efficacy of a New Enzyme Immunoassay for Creatine Kinase MB Isoenzyme, Clin. Chem 30: 1399-1401.
- 10) Abbott, L.B., Van Lente, F., 1985, Methods for Ck-MB Analysis Used to Monitor Cardiac Patients., J. Clin. Immunoassay 8: 147151.
- 11) Young, D., 1990, Effects of Drugs on Clinical Laboratory Tests. 3rd Edition, Washington, DC, American Association for Clinical Chemistry Press.



TOSOH CORPORATION
Bioscience Division
 2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan
 Phone: +81 3 6636 3734
 Fax: +81 3 6636 3627
 Website: www.tosoh.com

Supplied by

TOSOH BIOSCIENCE, INC.
 3600 Gantz Road,
 Grove City, OH 43123 USA
 Phone: +1 650 615 4970
 Fax: +1 650 615 0415
 Phone: (800) 248 6764
 Fax: (800) 685 7595
 Website: www.tosohbioscience.us

 European Conformity	 <i>In vitro</i> diagnostic medical device	 Consult instructions for use	 Temperature limitation
 Batch code / Lot number	 Manufacturer	 Authorized representative in the European Community	 Use by date
 Catalogue number / Part number	 Supplied by	 Sufficient for	 US: For prescription use only

AIA-PACK

CK-MB Calibrator Set

Intended Use

The AIA-PACK CK-MB Calibrator Set is intended for IN VITRO diagnostic use only for the calibration of the ST AIA-PACK CK-MB Assays.

Summary and Explanation

The AIA-PACK CK-MB Calibrator Set contains bovine serum with assigned levels of Creatine Kinase MB Isoenzyme. Calibration should be performed according to the schedule indicated in the AIA System Operator's Manual. The calibrators for use with the ST AIA-PACK CK-MB are prepared gravimetrically and are compared to internal reference standards.

Material Provided (Cat. No. 0020369)

2 x 1 mL	Calibrator No. 1	0	ng/mL
----------	------------------	---	-------

Bovine serum containing no detectable concentration of CK-MB (0 ng CK-MB/mL), and 0.1% sodium azide as preservative. (Ready for Use)

2 x 1 mL	Calibrator No. 2	10	ng/mL (approx.)
	No. 3	20	ng/mL (approx.)
	No. 4	100	ng/mL (approx.)
	No. 5	200	ng/mL (approx.)
	No. 6	500	ng/mL (approx.)

Bovine serum containing the assigned concentration of CK-MB (described on each vial) and 0.1% sodium azide as preservative. (Lyophilized)

Warnings and Precautions

- The AIA-PACK CK-MB 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 CK-MB calibrators, 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

- Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized calibrators accurately with 1.0 mL of 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.

Stability

When stored unopened and refrigerated at 2 - 8 °C, the AIA-PACK CK-MB 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 CK-MB Calibrator Set contains assigned concentrations of Creatine Kinase MB Isoenzyme. The assigned value is determined on a lot-to-lot basis and is designed to provide an assay calibration range of approximately 0 to 500 ng/mL of Creatine Kinase MB Isoenzyme. The calibrators for use with the ST AIA-PACK CK-MB are 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 CK-MB Calibrator Set is designed solely for use with AIA-PACK assay procedures.

References

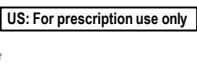
1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.



TOSOH CORPORATION
Bioscience Division
2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan
Phone: +81 3 6636 3734
Fax: +81 3 6636 3627
Website: www.tosoh.com

Supplied by

TOSOH BIOSCIENCE, INC.
3600 Gantz Road,
Grove City, OH 43123 USA
Phone: +1 650 615 4970
Fax: +1 650 615 0415
Phone: (800) 248 6764
Fax: (800) 685 7595
Website: www.tosohbioscience.us

 European Conformity	 <i>In vitro</i> diagnostic medical device	 Consult instructions for use	 Temperature limitation
 Batch code / Lot number	 Manufacturer	 Authorized representative in the European Community	 Use by date
 Catalogue number / Part number	 Supplied by	 Sufficient for	 US: For prescription use only

AIA-PACK

CK-MB Sample Diluting Solution

Intended Use

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

Summary and Explanation

The AIA-PACK CK-MB Sample Diluting Solution contains bovine serum with no detectable concentration of Creatine Kinase MB Isoenzyme (CK-MB). This Sample Diluting Solution is to be used only with samples that are being tested for CK-MB concentrations using the ST AIA-PACK CK-MB assays.

Material Provided (Cat. No. 0020569)

4 x 4 mL Sample Diluting Solution

Bovine serum containing no detectable concentration of Creatine Kinase MB Isoenzyme (0 ng CK-MB/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK CK-MB 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.
- Although material derived from human blood is not used for CK-MB 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

- The AIA-PACK CK-MB 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 CK-MB Sample Diluting Solution is stable until the expiration date on the label. After opening, 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.
- If a specimen is found to contain greater than the linearity limit of approximately 500 ng/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.
- The AIA 900 and AIA-2000 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
- The recommended dilution for serum containing greater than the concentration of the highest calibrator is 1:10 or 1:100. However, it is desirable to dilute the serum samples so that the diluted sample reads between 0.5 and 500 ng CK-MB/mL.

Results

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

Limitations

The AIA-PACK CK-MB Sample Diluting Solution 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.

**TOSOH CORPORATION****Bioscience Division**

2-2-1 Yaesu, Chuo-ku, Tokyo 104-0028 Japan

Phone: +81 3 6636 3734

Fax: +81 3 6636 3627

Website: www.tosoh.com**Supplied by****TOSOH BIOSCIENCE, INC.**

3600 Gantz Road,

Grove City, OH 43123 USA

Phone: +1 650 615 4970

Fax: +1 650 615 0415

Phone: (800) 248 6764

Fax: (800) 685 7595

Website: www.tosohbioscience.us

European Conformity

*In vitro* diagnostic medical device

Consult instructions for use



Temperature limitation



Batch code / Lot number



Manufacturer

Authorized representative
in the European Community

Use by date

Catalogue number
/ Part number

Supplied by



Sufficient for

US: For prescription use only