AIA-360 Assay Specifications
ST PA Test Code 028

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/mL
3	Cal 2	50 ng/mL (example.)
4		
5		
6		
7		
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/mL
11	Smpl. Vol	20
12	Dil. Vol	100
13	Assay L	0.05
14	Assay H	100.0
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	#PA
19	Calib. No	2
20	Calib. Mul	3
21	Calib. Equ	1
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 PA Test Code 028

No.	Item	Data
1	Code	28
2	ACT	1
3	Analyte	# PA
4	Lot	***Enter current cal. lot no.
5	CAL 1	0 ng/mL
6	CAL 2	50 ng/mL (example.)
7		
8		
9		
10		
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/mL
14	Decimal	2
15	Assay Low	0.05
16	Assay High	100.00
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.05
20	Reschedule High	100.00
21	Factor A	1
22	Factor B	0
23	Sample Volume	20
24	Diluent Volume	100
25	2 Step reagent dispensing volume	0
26	Calibration code	12
27	CAL. No.	2
28	CAL. MUL.	3
29	CAL. EQU.	1
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	12
39	DIL. NAME	PA
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 PA Test Code 028

No.	Item	Data
1	Unit	ng/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.05
6	Reschedule high	100.00
7	Assay range low	0.05
8	Assay range high	100.00
9	Specimen diluent code	12
10	Specimen diluent name	PA
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	1
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	50 ng/mL (example.)
24		
25		
26		
27		
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	20
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	1
49	Calibration Code Check	012
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

Prostate Specific Antigen
ST AIA-PACK PA

Caution: The sale and distribution of this device is restricted by United States federal law to, by, or on the order of a physician. In addition, the use of this device is restricted to, by, or on the order of a physician. Because of differences in reagent specificity and assay methods, the concentration of PSA in a given specimen may vary with devices from different manufacturers. Values obtained with different assay methods cannot be used interchangeably. It is mandatory that results reported by the laboratory to the physician include the identity of the assay used. If the assay method for PSA is changed during the course of monitoring patients with serial PSA levels, baseline values for the patients being serially monitored must be confirmed by additional sequential testing.

Name and Intended Use

ST AIA-PACK PA is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Prostate Specific Antigen (PSA) in human serum or heparinized plasma on Tosoh AIA analyzers. This device is indicated for the measurement of serum or plasma PSA in conjunction with Digital Rectal Examination (DRE) as an aid in the detection of prostate cancer (CaP) in men fifty years of age and older. Prostate biopsy is required for the diagnosis of cancer.

This device is further indicated for the serial measurement of PSA in human serum or plasma to be used as an aid in the management of patients with prostatic cancer.

Summary and Explanation of Test

Prostate Specific Antigen (PSA) was identified in 1977 by Wang, et al.[1] PSA is a single chain glycoprotein of approximately 34,000 daltons containing 7% carbohydrate.[2] Functionally and immunohistochemically, PSA is distinct from prostatic acid phosphatase (PAP)[3] and is confined to the cytoplasm of prostatic acinar cells and ductal epithelium.[4]

The presence of PSA has been demonstrated in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid as well as seminal plasma.[5] PSA is not present, however, in any other tissue from men, nor is it produced by cancers of the lung, colon, rectum, stomach, pancreas or thyroid.[6] Elevated serum PSA levels have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues.[3,7] Prostate cancer cannot be diagnosed until biopsy results confirm the presence of cancer cells. Studies indicate that PSA is an important tool in assessing the effect of therapy.[8] Especially in patients being treated with hormone therapy, concurrent serial determinations of PSA and PAP may provide added clinical value in monitoring patients with prostatic cancer.[9]

Digital rectal examination (DRE) is a widely used technique for detecting prostate cancer, but this procedure used by itself can miss significant numbers of prostatic cancers, especially organ confined tumors presenting in prostate locations difficult to palpate. The detection of these organ confined tumors is particularly important since an effective means of treatment of tumors found at this early stage currently exists. In addition, it has been clearly demonstrated that the incidence of prostate cancer increases with age. With the increases in life expectancy, it is even more critical that the disease be diagnosed early because more men will develop prostate cancer during their lifetime.

Since the mid-1980's, there has been a growing body of literature concerning the utility of Prostate Specific Antigen (PSA) for both monitoring and detection of prostate cancer (CaP). Catalona et al. evaluated the detection of prostate cancer in conjunction with DRE. Of the 6,630 men participating in the study, biopsies were performed on 1,167 men for a biopsy rate of 17.6%. To be eligible for biopsy in this study required a PSA value greater than 4.0 ng/mL or a suspicious DRE result. Prostate cancer was found in 264 of these patients (22.6%) a percentage that is lower than more recent studies involving serially accrued patients undergoing biopsy procedures. The general finding was that PSA enhances the ability of DRE and the detection of prostate cancer when used in combination.

Principle of the Assay

The ST AIA-PACK PA is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. PSA 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 PSA concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve. Due to the epitope sites used, the Tosoh ST AIA-PACK PA (PSA) has been shown to react in an equimolar fashion with both unbound PSA (free PSA) and that which is complexed to anti-chymotrypsin (PSA-ACT).

Material Provided (ST AIA-PACK PA, Cat. No. 0025263)

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

Plastic test cups containing lyophilized magnetic beads coated with anti-PSA mouse monoclonal antibody and mouse monoclonal antibody (to human PSA) 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 Prostate Specific Antigen analysis using the ST AIA-PACK PA (Cat. No. 0025263) on the Tosoh AIA systems. They are available separately from Tosoh.

Materials	Cat. No.
AIA-SYSTEMS:	
AIA-900	0022930
AIA-900 9tray Sorter	0022931
AIA-900 19tray Sorter	0022932
AIA-360	0019945
AIA-2000 ST	0022100
AIA-2000 LA	0022101
AIA-PACK:	
AIA-PACK Substrate Set II	0020968
AIA-PACK Substrate/Reconstituent	
AIA-PACK PA Calibrator Set	0020363
Calibrator Zero 0 ng/mL	
Positive 50 ng/mL (approx.)	
AIA-PACK PA Sample Diluting Solution	0020563
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 PA 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.

ST AIA-PACK PA 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 PA has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with PSA concentrations between 100 and 9,000 ng/mL will read > 100 ng/mL. The "hook effect" phenomenon may occur only at PSA concentrations > 9,000 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 PA	0025263
AIA PACK Calibrator Set	0020363
AIA PACK Sample Diluting Solution	0020563
AIA PACK Calibration Verification/Linearity Test Set	0020663
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 PA 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 is required for the 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.

Because there is controversy as to whether any prostatic manipulation affects the PSA results, samples should be drawn before any prostatic procedures such as DRE, prostatic massage and TRUS are performed.

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 20 µL.

Procedure

Reagent Preparation

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; mix thoroughly to dissolve.

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.

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.

Calibration

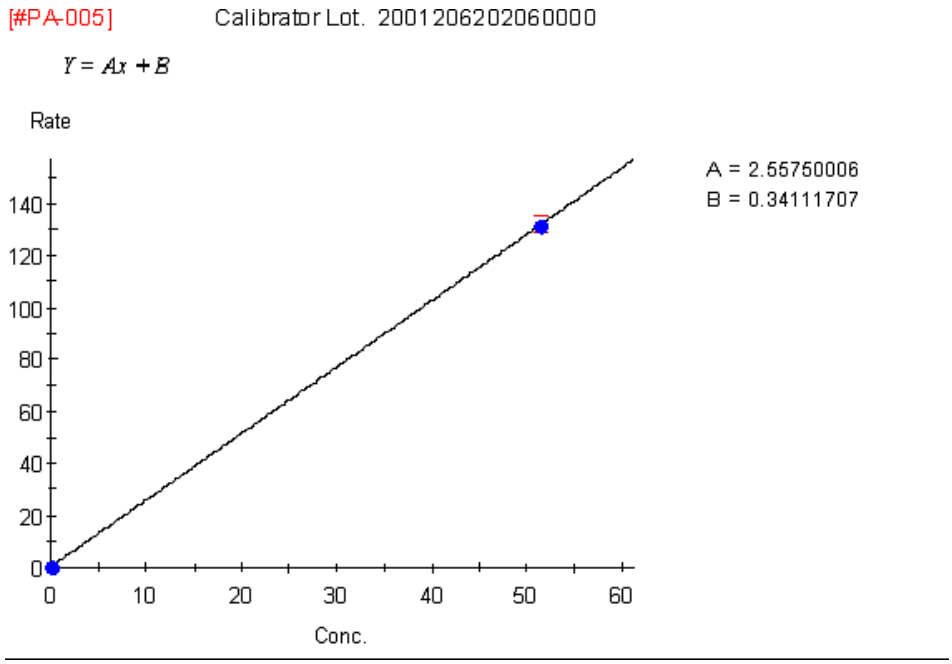
Calibration Curve

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

The calibration curve for the ST AIA-PACK PA 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.



Calibration Procedure

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

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

Calibrators for St AIA-PACK PA are provided ready to use. Tosoh recommends that all calibrators be run in triplicate.

Calibration Acceptability criteria

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 increaseas the concentration increases.

The replicate values should be within a 10% range.

Calibration Review and Acceptance

Review the calibration curve carefully, using the criteria listed above.

Edit the calibration if necessary, then accept the calibration.

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

Quality Control

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.

Quality Control Procedure

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.

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

Specimen Processing

Preparation

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

Assay Procedure

Ensure a sufficient quantity of ST AIA-PACK PA test cups for the number of samples to be run.

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 Prostate Specific Antigen concentration is found to be greater than the linearity limit of the assay 100 ng/mL, the specimen should be diluted with the PA Sample Diluting Solution and reassayed according to the Assay Procedure. The recommended dilution for samples containing greater than 100 ng/mL is 1:10 or 1:100. It is desirable to dilute the sample so that the diluted sample reads between 2 and 100 ng/mL. Dilutions may either be made manually or programmed into the analyzer. If automated dilutions are used, the dilution factor should be entered into the software. Refer to the AIA System Operator's Manual for further information on the dilution of specimens.
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 PA 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 028.

Calculation of Results

The AIA Systems perform all sample and reagent handling operations automatically. All the AIA Systems read the rate of fluorescence produced by the reaction and automatically convert the rate to Prostate Specific Antigen concentration in ng/mL.

For samples requiring dilution, the AIA-900 and AIA-2000 will automatically perform dilutions and calculate results if the dilution factors are entered into the software. 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 PA, the highest concentration of Prostate Specific Antigen measurable without dilution is 100 ng/mL, and the lowest measurable concentration is 0.05 ng/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 50 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, 100 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 or decreased PSA values.

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 corresponding 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.[10]

Results from the ST AIA-PACK PA assay should not be interpreted as being definitive for the presence or absence of prostate cancer. Patients with levels of PSA within the reference interval found in apparently healthy subjects may have prostate cancer; patients with levels exceeding those in the reference interval may be prostate cancer free. Results from the ST AIA-PACK PA should be interpreted in the light of other clinical findings and diagnostic procedures such as DRE. Biopsy of the prostate is currently the medically accepted standard used to confirm the presence/absence of prostate cancer.

The following data was collected using the AIA-PACK PA. The ST AIA-PACK PA demonstrates equivalent performance. (See Comparative Analysis.)

Reference Ranges

The interval given here was determined in serum samples from 863 apparently healthy male subjects.

Category	Men
Number of Samples (n)	863
Reference Interval	0 - 4.0 ng/mL

In this study, 98.7% of the healthy subjects had serum PSA concentrations less than or equal to 4.0 ng/mL. Results of this study are categorized below:

Category	Women	Men <40 years	Men ≥ 40 years
Number of Samples (n)	304	282	581
Mean (x)	0.0 ng/mL	0.63 ng/mL	1.08 ng/mL
SD	0.0 ng/mL	0.44 ng/mL	0.93 ng/mL
Range of Values	0.00 - 0.28 ng/mL	0.00 - 1.51 ng/mL	0.00 - 6.40 ng/mL
± 2 SD range		0.00 - 1.51 ng/mL	0.00 - 2.94 ng/mL

Expected Values for Management of Patients with Prostate Cancer

Distribution of Serum AIA-PACK PA Concentrations in Healthy Subjects, Malignant and Benign Disease States

	Number of patients	0 - 4.0 ng/mL	4.01- 10.0 ng/mL	10.01-20.0 ng/mL	20.01-40.0 ng/mL	>40.0 ng/mL
Healthy Subjects						
Men<40	282	99.6%	0.4%	0%	0%	0%
Men≥40	581	98.3%	1.7%	0%	0%	0%
Total Men	863	98.7%	1.3%	0%	0%	0%
Women	304	100.0%	0.0%	0%	0%	0%
Total	1167	99.1%	0.9%	0%	0%	0%
Malignant Diseases						
Prostate						
Stage A	120	62.5%	23.3%	10.8%	2.5%	0.8%
Stage B	159	49.1%	22.0%	17.6%	6.3%	5.0%
Stage C	136	39.7%	17.6%	14.0%	16.2%	12.5%
Stage D	167	21.6%	8.4%	8.4%	11.4%	50.3%
Total Prostate	582	41.8%	17.4%	12.7%	9.3%	18.8%
Gastrointestinal Males	41	92.7%	2.4%	0.0%	2.4%	2.4%
Gastrointestinal Females	21	100.0%	0.0%	0.0%	0.0%	0.0%
Genitourinary Males	64	87.5%	10.9%	1.6%	0.0%	0.0%
Genitourinary Females	21	100.0%	0.0%	0.0%	0.0%	0.0%
Mammary Males	1	0.0%	100.0%	0.0%	0.0%	0.0%
Mammary Females	60	100.0%	0.0%	0.0%	0.0%	0.0%
Pulmonary Males	37	91.9%	8.1%	0.0%	0.0%	0.0%
Pulmonary Females	24	100.0%	0.0%	0.0%	0.0%	0.0%
Other Males[A]	17	100.0%	0.0%	0.0%	0.0%	0.0%
Other Females[B]	5	100.0%	0.0%	0.0%	0.0%	0.0%
Nonmalignant Diseases						
Benign Prostate Hypertrophy Males	173	64.7%	23.7%	8.7%	2.3%	0.0%
Misc. Benign Genitourinary Males	23	82.6%	17.4%	0.0%	0.0%	0.5%
Misc. Benign Genitourinary Females	25	100.0%	0.0%	0.0%	0.0%	0.0%
Other Benign Males[C]	32	90.6%	9.4%	0.0%	0.0%	0.0%
Other Benign Females[D]	39	100.0%	0.0%	0.0%	0.0%	0.0%

2332 single specimens (patients) were analyzed for this distribution chart.

a. leukemia 1, parotid CA 1, liposarcoma and adrenal adenoma 1, kidney 2, testicular 6, pancreas 3, liver 3

b. pancreas 2, liver 2, ovarian 1

c. Pancreatitis 2,hepatitis 2,cirrhosis 4,asthma 1,cysts,mole,benign tumors (lung, ulcer, bowel)

d. pancreatitis 4, benign breast 4, hepatitis 4, cirrhosis 2, bronchitis 1, asthma 1, COPD 3, laryngitis, cysts, lipoma, thyroid adenoma, hyperthyroidism, hemangioma, benign tumors and colon, lung abscess, bronchitis, colitis, cholecystitis

Expected Values for Detection of Prostate Cancer

Catalona, et al. [11] conducted a multi-center, prospective clinical trial on men over the age of fifty years to test the effectiveness of PSA along with digital rectal examination (DRE) in detecting prostate cancer. This study showed that PSA measurements in conjunction with DRE were more effective in detecting prostate cancer than either DRE or PSA measurements alone. PSA elevations above 4.0 ng/mL detected 41% of cancers that DRE did not find. Conversely, DRE detected 21% of cancers that PSA, using a cutoff of 4.0 ng/mL, did not uncover.

Our studies with the AIA-PACK PA reached similar conclusions: PSA testing should be done in conjunction with DRE because used in tandem these tests detect a far greater number of prostate cancers than either test used alone.

A prospective study was performed using the AIA-PACK PA on the AIA-600 II. It included 510 men 50 years of age and older from 11 clinical sites referred to urologists for determination of the presence of prostate cancer with no previous history of either prostate disease or evaluation for prostate cancer. This study showed the significant association between biopsy result and PSA value as measured by AIA-PACK PA.

The following chart shows the distribution of disease by DRE and PSA result. Only 58 of the 185 Prostate Cancer patients were identified by DRE alone. The positive predictive value of a suspicious DRE in this study was 58.0%.

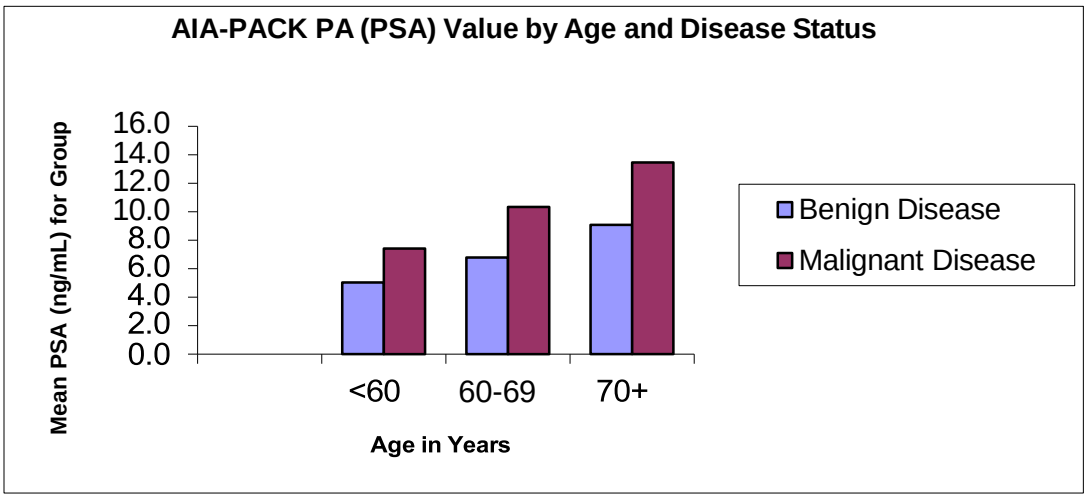
Distribution of Disease Status by PSA and DRE Result

DRE Result	PSA (ng/mL)	Biopsy – Malignant	Biopsy – Benign	Total
Suspicious	≥4.0	47	29	76
Suspicious	<4.0	11	13	24
Normal	≥ 4.0	112	216	328
Normal	<4.0	15	67	82
Total		185	325	510

When used without DRE and age, PSA at a cutoff of 4.0 ng/mL yielded a positive predictive value of 39.4%. Using AIA-PACK PA (PSA) at a cutoff of 4.0 ng/mL with the DRE increased the number of correctly identified patients to 170. The positive predictive value using both tests in tandem was 39.7%. The added value of PSA and DRE over DRE alone is 60.5% ((170-58)/185).

Of the 185 prostate cancers confirmed by biopsy in the above prospective study, Gleason grade scores ranged from 3 to 10. The median Gleason grade was 6.0 with a mean of 6.3. The mean value of PSA for men with a Gleason grade of 4 or less is lower than either those graded 5 – 7 or those grouped from 8 – 10 (5.7 ng/mL vs 10.3 ng/mL vs 20.1 ng/mL).

The chart below shows the mean value of PSA by age category and disease status. The results indicate a significant association between biopsy result, age and PSA value.



Note: Though our clinical studies clearly indicate that PSA and DRE together are useful in detecting a greater number of prostate cancers, negative results on either DRE or PSA or both do not preclude the presence of prostate cancer. Also, positive results on DRE and/or PSA do not confirm a diagnosis of prostate cancer. The results of a prostatic biopsy must be used to confirm a prostate cancer diagnosis.

Performance Characteristics

The following performance characteristics for ST AIA-PACK PA were determined using the Tosoh AIA-900 Automated Immunoassay Analyzer. All AIA systems demonstrate equivalent performance.

1) Accuracy

1a) Recovery:

Three serum pools were spiked with four different levels of PSA and assayed before and after spiking.

Sample	Initial Value (ng/mL)	PSA Added (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A1	0.917	68.75	69.67	70.05	100.5
	0.917	34.38	35.29	35.66	101.0
	0.917	17.19	18.11	17.93	99.0
Serum B1	0.961	68.75	69.72	67.84	97.3
	0.961	34.38	35.34	33.82	95.7
	0.961	17.19	18.15	18.10	99.7
Serum C1	2.189	68.75	70.94	71.33	100.5
	2.189	34.38	36.57	37.94	103.8
	2.189	17.19	19.38	19.86	102.5

1b) Dilution:

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

Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A2	none		89.63	100.0
	7.5/10	67.22	69.27	103.1
	5.0/10	44.81	45.07	100.6
	2.5/10	22.41	22.68	101.2
	1.0/10	8.96	9.18	102.4
Serum B2	none		89.34	100.0
	7.5/10	67.00	67.17	100.3
	5.0/10	44.67	45.27	101.3
	2.5/10	22.33	22.06	98.8
	1.0/10	8.93	8.85	99.1
Serum C2	none		86.18	100.0
	7.5/10	64.64	63.03	97.5
	5.0/10	43.09	40.97	95.1
	2.5/10	21.55	20.55	95.4
	1.0/10	8.62	8.02	93.0

1c) Comparative Analysis:

A group of well-characterized frozen retrospective samples were collected and analyzed. Results from the ST AIA-PACK PA assay (y) were compared to those obtained using the AIA-PACK PA assay (x). A total of 245 samples were tested in the study.

Slope 1.069 (95% CI: 1.062, 1.077)

y-intercept 0.079 (95% CI: -0.065, 0.224)

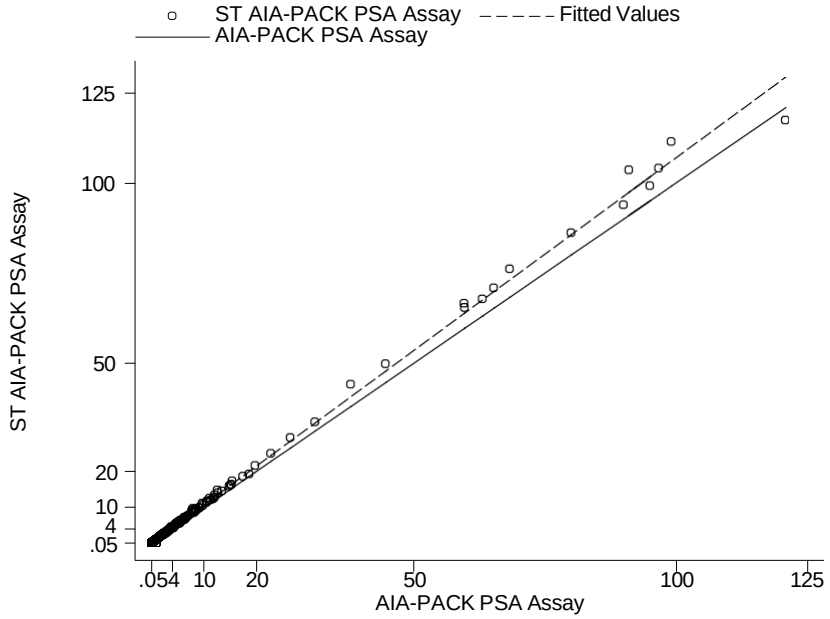
Correlation Coefficient 0.9949 Range of Samples (x) 0.05 - 100

Number of Samples 245

Figure 1

Comparative Analysis

ST AIA-PACK PA vs AIA-PACK PA (40 min)



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 calculated the coefficient of variation (CV).

Sample	Mean (ng/mL)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Serum A3	2.27	0.055	2.4
Serum B3	7.08	0.110	1.6
Serum C3	29.54	0.487	1.7

2b) Inter-assay precision

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

Sample	Number of Replicates	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A3	20	2.23	0.056	2.5
Serum B3	20	7.07	0.093	1.3
Serum C3	20	29.92	0.690	2.3

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)	Pooled SD (ng/mL)	Coefficient of Variation (%)
Serum A3	2.27	0.094	4.1
Serum B3	7.08	0.209	3.0
Serum C3	29.54	0.934	3.2

Specificity

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

Compound	Cross-reactivity (%)
PAP	0.06

Sensitivity

The minimal detectable concentration (MDC) of Prostate Specific Antigen is estimated to be 0.05 ng/mL. The MDC is defined as that concentration of PSA 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 16 mg/dL) and conjugated bilirubin (up to 18 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.

The following interference studies were performed on the AIA-PACK PA:

Chemotherapeutic agents: Therapeutic concentrations of the following chemotherapeutic agents added to serum samples which were then assayed, do not interfere with AIA-PACK PA Assay: Cisplatin, Bleomycin, Adriamycin, Cytoxan, Vincristine, Diethylstilbestrol, Methotrexate, 5-Fluorouracil and Mitomycin C.

A study of 53 men with no prior evidence of prostate disease who were regularly taking aspirin, anticoagulants, anti-hypertensives, anti-inflamatory drugs, antibiotics, hypo-glycemics and/or anti-arrhythmic medication was undertaken to determine whether these commonly prescribed medications would affect the blood levels of PSA. Levels of PSA as measured by the AIA-PACK PA were not affected by the use of these drugs in a prostate disease-free cohort.

The ST AIA-PACK PA demonstrates equivalent performance.

References

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4. Nadji, M., et al, 1981, Prostatic-Specific Antigen: An Immunohistologic Marker for Prostatic Neoplasms. Cancer 48:1229.
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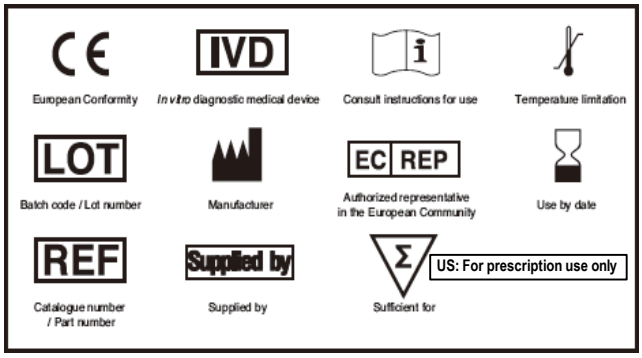


AIA-PACK
PA Calibrator Set

Intended Use

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

Summary and Explanation

The AIA-PACK PA Calibrator Set contains human serum with assigned levels of Prostate Specific Antigen. 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. 0020363)

2 x 1 mL	Zero Calibrator Human serum containing no detectable concentration of PSA (0 ng PSA/mL), and 0.1% sodium azide as preservative. (Ready for Use)
2 x 1 mL	Positive Calibrator Human serum containing the assigned concentration of PSA (approximately 50 ng PSA/mL, described on each vial) and 0.1% sodium azide as preservative. (Ready for Use)

Warnings and Precautions

- The AIA-PACK PA 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.
- 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 PA calibrators are provided ready to use.
- Bring calibrator to room temperature (18 - 25 °C) before 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 PA 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.

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 PA Calibrator Set contains assigned concentrations of Prostate Specific Antigen. 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 100.0 ng/mL of Prostate Specific Antigen. The calibrators in this set are prepared gravimetrically and 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.

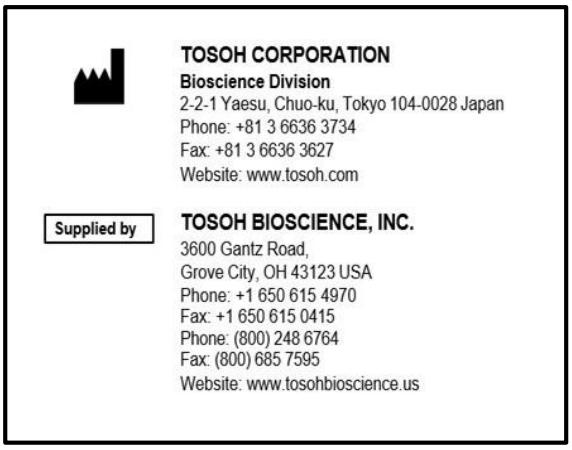
The replicate values should be within a 10% range.

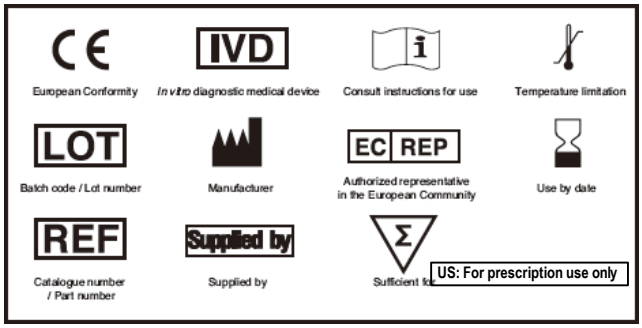
Limitations

The AIA-PACK PA 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
PA Sample Diluting Solution

Intended Use

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

Summary and Explanation

The AIA-PACK PA Sample Diluting Solution contains human serum with no detectable concentration of Prostate Specific Antigen (PSA). This Sample Diluting Solution is to be used only with samples that are being tested for Prostate Specific Antigen concentrations using the ST AIA-PACK PA Assay.

Material Provided (Cat. No. 0020563)

4 x 4 mL Sample Diluting Solution

Human serum containing no detectable concentration of Prostate Specific Antigen (0 ng PSA/mL), and 0.1% sodium azide as a preservative.

Warnings and Precautions

- The AIA-PACK PA 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 PA 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 PA 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.

1. If a specimen is found to contain greater than the linearity limit of 100 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.
2. 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.
3. The recommended dilution for specimen containing greater than 100 ng/mL is 1:10 or 1:100. However, it is desirable to dilute the samples that contain more than 100 ng PSA/mL so that the diluted sample reads between 2 and 100 ng PSA/mL.

Results

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

Limitations

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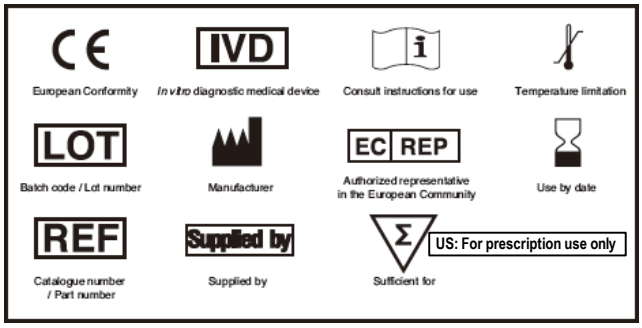
European Conformity
IVD
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
Supplied by
Sufficient for
US: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

AIA-PACK
PA Calibration Verification/Linearity Test Set

Intended Use

The AIA-PACK PA Calibration Verification/Linearity Test Set is intended for IN VITRO diagnostic use only for the verification of the calibration and linearity of the ST AIA-PACK PA Assays.

Summary and Explanation

The AIA-PACK PA Calibration Verification/Linearity Test Set can be used as part of a quality assurance program to assist in complying with various regulatory requirements under which an institution may operate. Specific guidelines for the acceptability of data are determined by the institution in conformance with these requirements. Good laboratory practices stipulate using different materials for calibration and quality control purposes. Calibration Verification Materials should be analyzed as unknown test samples according to the directions stated in the AIA System Operator's Manual.

Material Provided (Cat. No. 0020663)

2 x 4 mL	Sample Diluting Solution (SDS) Human serum containing no detectable concentration of PSA (0 ng PSA/mL), and 0.1% sodium azide as preservative. (Ready to Use)
2 x 2 mL	Calibration Verification Material (CVM) Human serum containing the assigned concentration of PSA (approximate upper linearity limit of 100 ng PSA/mL, described on each vial) and 0.1% sodium azide as preservative. (Ready to Use)

Warnings and Precautions

- The AIA-PACK PA Calibration Verification/Linearity Test 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 materials, 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 Calibration Verification Material and Sample Diluting Solution are provided ready to use.
- The materials should be at room temperature (18 - 25 °C) prior to use.
- Always store the Calibration Verification/Linearity Test 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 PA Calibration Verification/Linearity Test Set is stable until the expiration date on the label. After opening, the materials should be used within 24 hours.

Procedure

The frequency of performing calibration verification and linearity of the ST AIA-PACK PA assay is established by each institution in accordance with its quality assurance program and appropriate regulatory requirements. Refer to the ASSAY PROCEDURE in the AIA-PACK section of this analyte application document. For additional procedural instructions regarding auto dilution, refer to the AIA System Operator's Manual.

Manual Dilutions

1. Make the desired dilutions of the Calibration Verification Material with Sample Diluting Solution and mix well. A sufficient amount should be made to assay each diluted sample in triplicate.
2. Program the instrument to run each of the prepared dilutions three times.
3. Add the appropriate amounts of each diluted sample to sample cups (refer to the instrument worklist for the amount required in each sample cup).
4. Verify the appropriate amount of test cups for the assay.
5. Load sample cups on the instrument as instructed in the AIA System Operator's Manual.
6. Start the assay.
7. Record the values obtained on the appropriate forms and calculate the desired statistics.

Assignment of Values

The Calibration Verification Material contains an assigned concentration of Prostate Specific Antigen. The assigned value is determined on a lot-to-lot basis and is designed to approximate the upper linear range of the assay. The Calibration Verification Material in this set is prepared gravimetrically and compared to internal reference standards.

Results

Make a determination of the acceptability of the data according to the specific guidelines established by your institution which satisfy the regulatory requirements under which your institution operates. If results do not meet your specifications, please initiate corrective action, as appropriate, or contact Tosoh Bioscience, Inc. for assistance.

Retain test records in the laboratory in the designated location for future reference.

Limitations

The AIA-PACK PA Calibration Verification/Linearity Test Set is designed solely for use with AIA-PACK assay procedures. The AIA-PACK PA Calibration Verification/Linearity Test Set will only verify linearity between the established sensitivity of the assay and the value of analyte level listed on the Calibration Verification Material label.

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.