AIA-360 Assay Specifications
ST AIA-PACK PROG III Test Code 123

No.	Item	Data
1	Calib. Req	Show
2	Cal 1	0 ng/mL
3	Cal 2	0.5 ng/mL (example.)
4	Cal 3	1.5 ng/mL (example.)
5	Cal 4	5.0 ng/mL (example.)
6	Cal 5	15 ng/mL (example.)
7	Cal 6	45 ng/mL (example.)
8	Cal Lot L	
9	Cal Lot R	
10	Unit	ng/mL
11	Smpl. Vol	30
12	Dil. Vol	100
13	Assay L	0.10
14	Assay H	40
15	Ref. L	
16	Ref. H	
17	Decimal	2
	Visible only in Test Mode	
18	Test Name	#PR-3
19	Calib. No	6
20	Calib. Mul	3
21	Calib. Equ	6
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-600II Assay Specifications
ST AIA-PACK PROG III Test Code 123

Screen	Item	Data
Screen 1		
	Lot	***Enter current cal. lot no.
	Cal 1	0 ng/mL
	Cal 2	0.5 ng/mL (example.)
	Cal 3	1.5 ng/mL (example.)
	Cal 4	5.0 ng/mL (example.)
	Cal 5	15 ng/mL (example.)
	Cal 6	45 ng/mL (example.)
Screen 2		
	Name	#PR-3
	Unit	ng/mL
	Sample	30
	Dil	100
	2Reag	0
	Code	0
	Assay Range Low	0.10
	Assay Range High	40
	Reference Range Low	
	Reference Range High	
	DP (No. of decimal points)	2
Screen 3		
	Code	123
	No. (Calibrators)	6
	Mul. (Replicates)	3
	Equ	6
	CV (Calibration curve stability)	90
	STAT (Analyte status)	0
	PRCL (Assay Protocol)	1
Screen 4		
	Dilution Factors:	
	SP1 (Specimen 1)	1
	SP2 (Specimen 2)	1
	CAL	1
	CTRL	1
	CODE (SDS code)	123
	PR (Dilution mode)	3
	Pretreatment:	
	SMPL (Pretreatment sample volume)	0
	VOL1 (Pretreatment 1 volume)	0
	VOL2 (Pretreatment 2 volume)	0.00
	CODE (Pretreatment code)	0.00

AIA-900 Assay Specifications
ST AIA-PACK PROG III Test Code 123

No.	Item	
1	Code	123
2	ACT	1
3	Analyte	#PR-3
4	Lot	***Enter current cal lot no.
5	CAL 1	0 ng/mL
6	CAL 2	0.5 ng/mL (example.)
7	CAL 3	1.5 ng/mL (example.)
8	CAL 4	5.0 ng/mL (example.)
9	CAL 5	15 ng/mL (example.)
10	CAL 6	45 ng/mL (example.)
11	Cal lot L	
12	Cal lot R	
13	Unit	ng/mL
14	Decimal	2
15	Assay Low	0.1
16	Assay High	40
17	Reference Low	
18	Reference High	
19	Reschedule Low	0.1
20	Reschedule High	40
21	Factor A	1
22	Factor B	0
23	Sample Volume	30
24	Diluent Volume	100
25	2 Step reagent dispensing volume	0
26	Calibration code	123
27	CAL. No.	6
28	CAL. MUL.	3
29	CAL. EQU.	6
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	1
36	DIL. AH.	5
37	DIL. CALC.	1
38	DIL. CODE	123
39	DIL. NAME	#PR-3
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-1800 Assay Specifications
ST AIA-PACK PROG III Test Code 123

No.	Item	
1	Unit	ng/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.10
6	Reschedule high	40
7	Assay range low	0.10
8	Assay range high	40.00
9	Specimen diluent code	123
10	Specimen diluent name	#PR-3
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	1
15	Default multiplier for >H	4
16	Factor A	1.0
17	Factor B	0.0
18	Calibration code	6
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	0.5 ng/mL (example.)
24	Cal - 03	1.5 ng/mL (example.)
25	Cal - 04	5.0 ng/mL (example.)
26	Cal - 05	15 ng/mL (example.)
27	Cal - 06	45 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	30
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	123
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

AIA-2000 Assay Specifications
ST AIA-PACK PROG III Test Code 123

No.	Item	
1	Unit	ng/mL
2	Decimal places	2
3	Reference low	
4	Reference high	
5	Reschedule low	0.10
6	Reschedule high	40
7	Assay range low	0.10
8	Assay range high	40
9	Specimen diluent code	123
10	Specimen diluent name	#PR-3
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	1
15	Default multiplier for >H	4
16	Factor A	1.0
17	Factor B	0.0
18	Calibration code	6
19	Calibrator replicates	3
20	Calibrator lot	***Enter current cal. lot no.
21	Calibrator Concentration	
22	Cal - 01	0 ng/mL
23	Cal - 02	0.5 ng/mL (example.)
24	Cal - 03	1.5 ng/mL (example.)
25	Cal - 04	5.0 ng/mL (example.)
26	Cal - 05	15 ng/mL (example.)
27	Cal - 06	45 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	30
39	Diluent volume	100
40	Conjugate volume	0
41	Diluted conjugate volume	0
42	Pretreatment	No
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	1.0
49	Calibration Check Code	123
50	Virtual Concentration	0.0
51	Graph Origin	0.0
52	Calculation with Dilution Factor	Yes

PROGESTERONE
ST AIA-PACK PROG III

Name and Intended Use

ST AIA-PACK PROGIII is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of progesterone in human serum or heparinized plasma on TOSOH AIA System Analyzers.

Summary and Explanation of Test

Progesterone (pregn-4-ene-3, 20-dione) is a steroid hormone produced mainly by the ovary and adrenal cortex in females and, in lesser amounts, by the adrenal cortex in males (1,2). Progesterone is secreted in small amounts by the ovaries during the follicular phase of the menstrual cycle and increases sharply following ovulation and corpus luteum development (3). Unless pregnancy occurs, a steep decline to follicular levels occurs prior to the next menstrual cycle (4). This pattern provides the rationale for the use of serum progesterone measurement as a reliable method for ovulation detection (5).

Daily progesterone levels are considered the most accurate means for documenting luteal phase defect *(6)*. Measurement of progesterone in blood can also be used to detect and evaluate patients at risk for abortion during the early weeks of gestation *(7,8)*.

Principle of the Assay

The ST AIA-PACK PROGIII is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK PROGIII test cups. Progesterone present in the test sample competes with enzyme-labeled progesterone for a limited number of binding sites on a progesterone-specific antibody immobilized on magnetic beads. The beads are washed to remove the unbound enzyme-labeled progesterone and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled progesterone that binds to the beads is inversely proportional to the progesterone concentration in the test sample. A standard curve using a range of known standard concentrations is constructed and unknown progesterone concentrations are calculated using this curve.

Material Provided

ST AIA-PACK PROGIII (Cat. No. 025240)

Plastic test cups containing lyophilized twelve magnetic beads with anti-progesterone sheep monoclonal antibody and 50 µL of progesterone conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Materials Required But Not Provided

The following materials are required to perform progesterone analysis using the ST AIA-PACK PROGIII (Cat. No. 025240) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials		**Cat. No.**
AIA-1800 ST		019836
AIA-1800 LA		019837
AIA-2000 ST		022100
AIA-2000 LA		022101
AIA-600 II		019014
AIA-600 II BCR		019328
AIA-900		022930
AIA-360		019945
AIA-PACK SUBSTRATE SET II		020968
AIA-PACK SUBSTRATE REAGENT II		
AIA-PACK SUBSTRATE RECONSTITUENT II		
ST AIA-PACK PROGIII CALIBRATOR SET		025340
ST AIA-PACK PROGIII CALIBRATOR (1)	0 ng / mL	
ST AIA-PACK PROGIII CALIBRATOR (2)	0.5 ng / mL (approx.)	
ST AIA-PACK PROGIII CALIBRATOR (3)	1.5 ng / mL (approx.)	
ST AIA-PACK PROGIII CALIBRATOR (4)	5.0 ng / mL (approx.)	
ST AIA-PACK PROGIII CALIBRATOR (5)	15 ng / mL (approx.)	
ST AIA-PACK PROGIII CALIBRATOR (6)	45 ng / mL (approx.)	
ST AIA-PACK PROGIII SAMPLE DILUTING SOLUTION		025540
AIA-PACK WASH CONCENTRATE		020955
AIA-PACK DILUENT CONCENTRATE		020956
SAMPLE CUPS		018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP		020970
AIA-PACK SAMPLE TREATMENT CUP		020971
Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000 :		
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 characterized based strictly on TOSOH materials.

Warnings and Precautions

- The ST AIA-PACK PROGIII 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 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 PROGIII 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.
- The material derived from human origin 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 origin, please use standard laboratory safety procedures in handling all specimens and controls.
- Do not use beyond the expiration date.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- After opening, the vial of ST AIA-PACK PROGIII SAMPLE DILUTING SOLUTION should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent and could give rise to erroneous results.
- The remaining sample diluting solution after use should not be mixed with another vial but be discarded to avoid contamination.
- 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.

Storage and Stability

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

Materials	Cat. No.
2 - 8 °C:	
ST AIA-PACK PROGIII	025240
ST AIA-PACK PROGIII CALIBRATOR SET	025340
ST AIA-PACK PROGIII SAMPLE DILUTING SOLUTION	025540
AIA-PACK SUBSTRATE SET II	020968
AIA-PACK WASH CONCENTRATE	020955
AIA-PACK DILUENT CONCENTRATE	020956
18 - 25 °C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	020970
AIA-PACK SAMPLE TREATMENT CUP	020971

When stored over night at 2 - 8 °C, the test cups can be used for up to 30 days (30 cycles of 8 hours on board and 16 hours in the refrigerator). Once the aluminum pouch is opened, the test cups must be used within 30 days.

ST AIA-PACK PROGIII CALIBRATOR SET must be kept tightly sealed and refrigerated at 2 - 8 °C. After opening, the calibrators should be used within 1 day.

When stored over night at 2 - 8 °C, the ST AIA- PACK PROGIII Sample Diluting Solution can be used for up to 9 days (9 cycles of 8 hours on board and 16 hours in the

refrigerator). The sample diluting solution can be used for 90 days, provided that: 1) it is used for manual dilutions only, and 2) the vials are kept tightly sealed and refrigerated immediately after use. The sample diluting solution should not be used beyond 90 days after opening, even if it is sealed and stored in the refrigerator.

Specimen Collection and Handling

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

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.

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

Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.

Samples containing inhibitors of alkaline phosphatase may cause erroneous results.

Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.

Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.

Samples may be stored at 2 - 8 °C for up to 7 days prior to analysis. If the analysis cannot be done within 7 days, 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, bring frozen samples to 18 - 25 °C slowly and mix gently.

The sample required for analysis is 30 µL.

Procedure

1) Reagent Preparation

For the AIA-360, AIA-600 II, AIA-900, AIA-1800, and AIA-2000, please refer to their Operator's Manual for detailed instructions.

A. Substrate Solution

Bring all reagents to 18 - 25 °C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the AIA-PACK SUBSTRATE REAGENT II (Lyophilized), mix thoroughly to dissolve the solid material.

B. Wash Solution

Add the entire contents of the AIA-PACK 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.

C. Diluent

Add the entire contents of the AIA-PACK 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

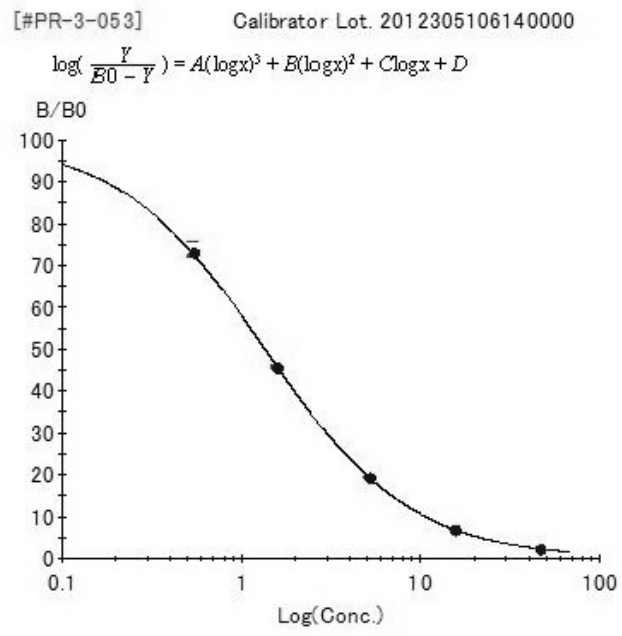
A. Calibration Curve

The calibrators for use with the ST AIA-PACK PROGIII were referred to USP (United States Pharmacopeia) Standard.

The calibration curve for ST AIA-PACK PROGIII is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and Tosoh 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 when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the Tosoh AIA System Operator's Manual.

A sample calibration curve from AIA-2000 follows and shows the algorithm used for calculating results.



B. Calibration Procedure

1. Refer to the appropriate Tosoh AIA System Operator's Manual for the procedural instructions.
2. Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
3. The ST AIA-PACK PROGIII CALIBRATOR SET is provided ready for use.
4. Tosoh recommends that all calibrators be run in triplicate.

C. Calibration Acceptability Criteria

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

D. 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.

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

3) Quality Control

A. Commercially Available Controls

Commercially available controls shall be run at least once per day. It is recommended that at least two 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.

B. Quality Control Procedure

1. Assay quality control specimens as instructed in the specific Operator's Manual for your analyzer. In addition, refer to the Tosoh 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. Follow federal, state and local guidelines for testing quality control materials.

4) Specimen Processing

A. Preparation

Following the specific instructions in the Operator's Manual for the analyzer, place samples on the instrument appropriately. Barcoded primary tubes as well as sample cups can be run on the AIA-360, AIA-600 II, AIA-900, AIA-1800, and AIA-2000.

B. Assay Procedure

1. Ensure a sufficient quantity of ST AIA-PACK PROGIII 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-600 II, AIA-900, AIA-1800 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 progesterone concentration is found to be greater than the upper limit of the assay range, 40 ng/mL, the specimen should be diluted with the ST AIA-PACK PROGIII SAMPLE DILUTING SOLUTION and reassayed according to the Assay Procedure. The recommended dilution for specimens containing greater than 40 ng/mL is 4 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 0.1 and 40.0 ng/mL. The dilution factor should be entered into the software. For further information on the dilution of specimens, refer to the Tosoh AIA System Operator's Manual.
4. The Tosoh AIA System Analyzers can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK PROGIII test cups can be used during the same run.
5. If the assay specifications for this test are not ready in the system software, the specifications must be entered under test code 123.

Calculation of Results

The Tosoh AIA System Analyzers perform all sample and reagent handling operations automatically. The Tosoh AIA System Analyzers read the rate of fluorescence produced by the reaction and automatically convert the rate to progesterone concentration in ng/mL.

For samples requiring dilution, the AIA-600 II, AIA-900, AIA-1800 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 Tosoh AIA System 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 shall be assayed according to the local regulations.

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

After calibration, two levels of the internal control are run in order to accept the calibration curve. The two levels of controls are repeated 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 shall be run in order to verify the overall performance of the Tosoh AIA System Analyzers.

If one or more control value(s) is out of the acceptable range, it is necessary to

investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the strict 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 PROGIII, the highest measurable concentration of progesterone in specimens without dilution is 40 ng/mL, and the lowest measurable concentration in specimens is 0.1 ng/mL (assay sensitivity).

Although the approximate value of the highest calibrator is 45 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, 40 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.

Heterophile antibodies are known to interfere in assays of this type. ST AIA-PACK PROGIII has been designed to minimize the effects of heterophile antibodies, but interference from high titers cannot be ruled out.

Samples from patients who had an injection of fluorescein, which is used in fluorescein fundus angiography, may cause false results.

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 *(9)*.

Reference Ranges

The interval given here was determined in serum samples from 260 apparently healthy American and Asian individuals. European studies showed that these reference values are also applicable for a European population.

Category	n	ranges (ng/mL)
Male	46	</= 0.88
Female		
Follicular phase	78	0.11 -- 0.95
Luteal phase	44	1.65 – 22.04
Postmenopausal females	38	</= 0.90
Pregnant females		
First trimester	42	3.24 – 60.54
Second trimester	40	21.52 – 104.58
Third trimester	40	66.52 – 367.64

Performance Characteristics

The following performance characteristics were determined using Tosoh AIA-2000 Automated Immunoassay Analyzer. All Tosoh AIA System Analyzers demonstrate equivalent performance.

1. Accuracy

1a) Recovery

Three serum samples and three heparinized plasma samples were spiked with three different concentration levels of PROG III and assayed before and after spiking.

Sample	Prog Value (ng/mL)	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Value (ng/mL)	Recovery %
Serum A1	0.342	6.65	6.99	6.78	97.1
	0.342	13.3	13.6	13.2	96.9
	0.342	26.6	26.9	28.8	106.8
Serum B1	0.465	6.65	7.11	6.93	97.4
	0.465	13.3	13.8	13.1	95.5
	0.465	26.6	27	27.9	103.2
Serum C1	0.512	6.65	7.16	7	97.8
	0.512	13.3	13.8	13.7	99.6
	0.512	26.6	27.1	28	103.2
Plasma A1	0.31	6.65	6.96	7.09	102
	0.31	13.3	13.6	13.4	98.8
	0.31	26.6	26.9	28.8	107
Plasma B1	0.343	6.65	6.99	6.37	91.2
	0.343	13.3	13.6	12.6	92.7
	0.343	26.6	26.9	26	96.6
Plasma C1	0.301	6.65	6.95	6.58	94.7
	0.301	13.3	13.6	12.7	93.8
	0.301	26.6	26.9	27.2	101.2

1b) Dilution

Three serum samples and three heparinized plasma samples containing high concentrations of progesterone were serially diluted with the ST AIA-PACK PROGIII SAMPLE DILUTING SOLUTION and assayed.

Sample	Dilution Factor	Expected Value (ng/mL)	Measured Value (ng/mL)	Percent Recovery (%)
Serum A2	none		30.8	
	7.5/10	23.1	23.4	101.4
	5.0/10	15.4	15.3	99.3
	2.5/10	7.7	7.82	101.6
	1.0/10	3.08	3.28	106.5
Serum B2	none		21.9	
	7.5/10	16.4	16.1	98.4
	5.0/10	10.9	10.5	95.9
	2.5/10	5.47	5.34	97.6
	1.0/10	2.19	2.26	103.4
Serum C2	none		32.4	
	7.5/10	24.3	23.4	96.4
	5.0/10	16.2	15.5	95.4
	2.5/10	8.1	7.82	96.5
	1.0/10	3.24	3.53	109.1
Plasma A2	none		33.3	
	7.5/10	25	24.6	98.4
	5.0/10	16.7	15.9	95.5
	2.5/10	8.32	7.81	93.8
	1.0/10	3.33	3.2	96.2
Plasma B2	none		33.3	
	7.5/10	25	24.5	97.9
	5.0/10	16.7	15.8	94.6
	2.5/10	8.33	8.04	96.5
	1.0/10	3.33	3.39	101.8
Plasma C2	none		22.9	
	7.5/10	17.2	17.7	102.7
	5.0/10	11.5	11.2	97.4
	2.5/10	5.73	6.14	107.2
	1.0/10	2.29	2.2	96

1c) Linearity

The linearity for ST AIA-PACK PROGIII was determined, based on guidance from CLSI Protocol EP06-A. The linearity was measured on the AIA-2000 instrument and has been demonstrated to be linear from 0.1 to 40 ng/mL.

2. Precision

Within run precision was determined using six 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).

2a) Intra-assay precision

Intra-assay (within run) Precision

Sample	Mean (ng/mL)	Pooled Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A3	2.26	0.0469	2.1
Serum B3	10.5	0.255	2.4
Serum C3	31.4	0.662	2.1
Heparinized Plasma A3	2.20	0.0441	2.0
Heparinized Plasma B3	11.3	0.214	1.9
Heparinized Plasma C3	33.3	0.937	2.8

2b) Total precision

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

Total Precision

Sample	Mean (ng/mL)	Pooled Standard Deviation (ng/mL)	Coefficient of Variation (%)
Serum A3	2.26	0.0654	2.9
Serum B3	10.5	0.298	2.8
Serum C3	31.4	0.959	3.1
Heparinized Plasma A3	2.20	0.0542	2.5
Heparinized Plasma B3	11.3	0.252	2.2
Heparinized Plasma C3	33.3	1.13	3.4

3. Correlation

The correlations between ST AIA-PACK PROG (x) and ST AIA-PACK PROGIII (y) were carried out using (1) 104 serum and (2) 306 heparinized plasma patient specimens, respectively.

	(1)	(2)
Slope	0.94	0.91
y-Intercept	0.177	0.089
Correlation Coefficient	0.984	0.997
Number of Samples	624	306

4. Specificity

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

Substance	Concentration added (ng/mL)	Cross-reactivity (%)
17α-hydroxyprogesterone	1,000	0.16
11-deoxycorticosterone	1,000	3.08
Pregnenolone	1,000	0.26
Corticosterone	1,000	0.97
5β-pregnan-3,20-dione	1,000	0.26
Testosterone	1,000	0.08
Cortisol	1,000	0.001
5α-pregnan-3,20-dione	1,000	0.62
DHEA-S	5,000	0.001

5. LoB, LoD and Functional Sensitivity

Limit of Blank

The limit of blank for ST AIA-PACK PROG III was determined, according to CLSI guideline EP17-A2. The calibrator represented the blank sample and was measured in 60 replicates. The LoB estimate was calculated for each reagent lot. The maximum LoB was estimated to be 0.03 ng/mL across 2 reagent lots, and was used to calculate the LoD estimates.

Limit of Detection

The limit of detection for ST AIA-PACK PROG III was determined, according to CLSI guideline EP17-A2. The blank sample was measured in 60 replicates. Five low level samples were measured in 12 replicates each. As a result, the limit of detection for ST AIA-PACK PROGIII was estimated to be 0.06 ng/mL.

Functional Sensitivity

The functional sensitivity for ST AIA-PACK PROG III was determined using low level samples (10) prepared by dilution of specimens with known progesterone concentrations. The samples were assayed in replicates of 2 over 3 days on 1 instrument for a total of 12 replicates per sample. Based on the imprecision profile, functional sensitivity was 0.20 ng/mL and 0.09 ng/mL at 10%CV and 20%CV, respectively.

6. Interference

Interference is defined, for the purposes of this study, with recovery outside of 10% of the known concentration of the specimen after the following substances are added to human specimens.

- Hemoglobin (up to 460 mg/dL), free bilirubin (up to 16 mg/dL) and conjugated bilirubin (up to 19 mg/dL) do not interfere with the assay.
- Lipemia, as indicated by triglyceride concentration (up to 160 mg/dL) does not interfere with the assay.
- Protein, as indicated by added albumin (up to 0.5 g/dL), does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.

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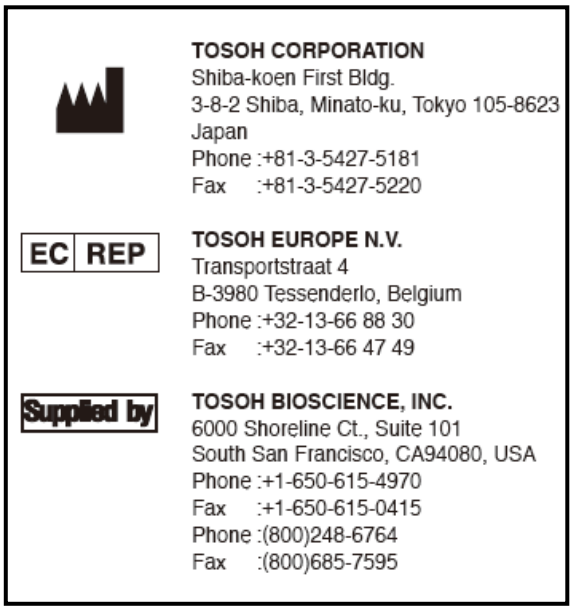
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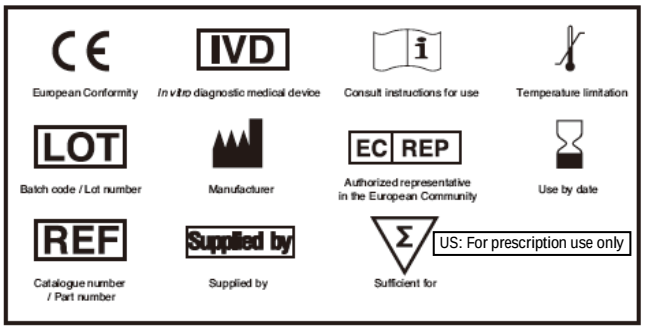
European Conformity
IVD
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
Sufficient for
US: For prescription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

AIA-PACK
PROGESTERONE III CALIBRATOR SET

Intended Use

The ST AIA-PACK PROG III Calibrator Set is intended for In Vitro Diagnostic Use Only for the calibration of the ST AIA-PACK PROG III assay.

Summary and Explanation

The ST AIA-PACK PROG III Calibrator Set contains bovine protein matrix with assigned levels of progesterone. Calibration should be performed according to the schedule indicated in the Tosoh AIA System Operator's Manual.

Material Provided (Cat. No. 025340)

2 x 1 mL ST AIA-PACK PROG III Calibrator (1)	0	ng/mL
Bovine protein matrix containing no detectable concentration of progesterone with sodium azide as a preservative. (Ready to Use)		
2 x 1 mL ST AIA-PACK PROG III Calibrator (2)	0.5	ng/mL (approx.)
ST AIA-PACK PROG III Calibrator (3)	1.5	ng/mL (approx.)
ST AIA-PACK PROG III Calibrator (4)	5.0	ng/mL (approx.)
ST AIA-PACK PROG III Calibrator (5)	15	ng/mL (approx.)
ST AIA-PACK PROG III Calibrator (6)	45	ng/mL (approx.)
Bovine protein matrix containing the assigned concentration of progesterone (described on each vial) with sodium azide as a preservative. (Ready to Use)		

Warnings and Precautions

- The ST AIA-PACK PROG III Calibrator Set is intended for in vitro diagnostic use only.
- For prescription use only.
- Inspect the packaging and the exterior of the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- This material 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.
- The calibrator material has been tested by FDA-approved method and found negative for the presence of HBsAg and antibody to HIV-1 and HCV. Because no

test method can offer complete assurance that the 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.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.

Preparation and Storage

Bring the calibrators to 18 - 25 °C for use. Always store the ST AIA-PACK PROG III 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 Calibrator Set is stable until the expiration date on the label. The calibrators should be used within 24 hours (1 day) of opening provided the vials are kept tightly sealed and refrigerated at 2 - 8 °C.

Procedure

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

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

Assignment of Values

The ST AIA-PACK PROG III Calibrator Set contains assigned concentrations of progesterone. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 0.1 to 40 ng/mL of progesterone. The calibrators for use with the ST AIA-PACK PROG III were referred to through the USP (United States Pharmacopeia) Standard (Lot #I1J239).

Results

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

The replicate values should be within a 10 % range.

Limitations

The ST AIA-PACK PROG III Calibrator Set is designed solely for use with ST AIA-PACK PROG III assay procedures. Although the approximate value of the highest calibrator is 45 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, 40 ng/mL.

References

1. AIA Analyte Application Manual (AAM) CD. Tosoh Bioscience, Inc., South San Francisco, CA.
2. AIA System Operator's Manual. Tosoh Bioscience, Inc., South San Francisco, CA.

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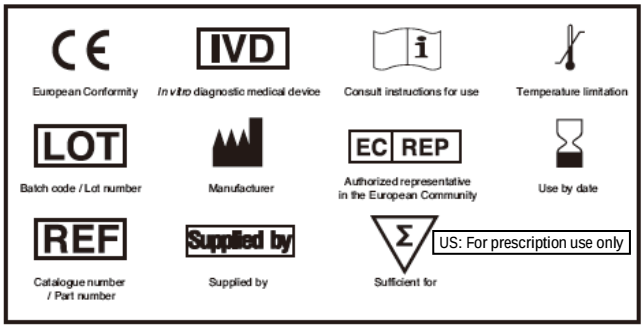


AIA-PACK
PROGESTERONE III SAMPLE DILUTING SOLUTION

Intended Use

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

Summary and Explanation

The AIA-PACK PROG III Sample Diluting Solution contains human serum with no detectable concentration of progesterone. This Sample Diluting Solution is to be used only with samples that are being tested for progesterone concentrations using the ST AIA-PACK PROG III assay.

Material Provided (Cat. No. 025540)

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

Warnings and Precautions

- The AIA-PACK PROG III 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 approved 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 PROG III 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 overnight at 2 - 8 °C, the AIA-PACK PROG III Sample Diluting Solution can be used for up to 9 days (9 cycles of 8 hours on board and 16 hours in the refrigerator). The sample diluting solution can be used for 90 days, provided that: 1) it is used for manual dilutions only, and 2) the vials are kept tightly sealed and refrigerated immediately after use. The sample diluting solution should not be used beyond 90 days after opening, even if it is sealed and stored in the refrigerator.

Procedure

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

1. If a specimen is found to contain greater than the linearity limit of approximately 45 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-600 II, AIA 900, AIA-1800 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 specimen containing greater than 40 ng/mL is 1:5. However, it is desirable to dilute the samples that contain more than 40 ng/mL so that the diluted sample reads between 2.0 and 20 ng/mL.

Results

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

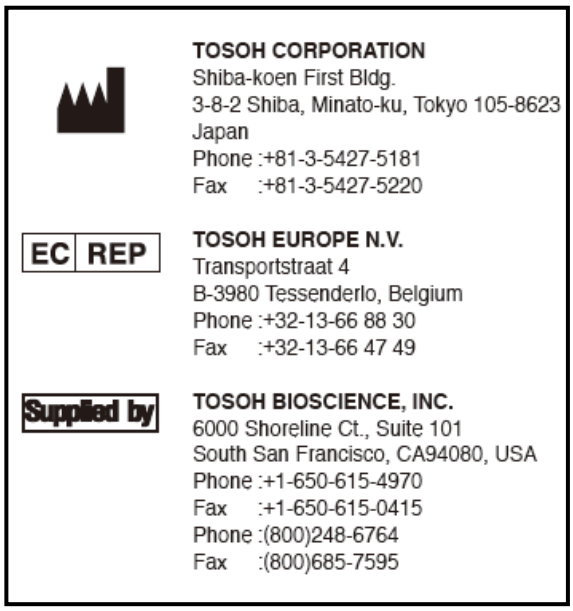
Limitations

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

References

1. AIA Analyte Application Manual (AAM) CD. Tosoh Bioscience, Inc., South San Francisco, CA.

2. AIA System Operator's Manual. Tosoh Bioscience, Inc., South San Francisco, CA.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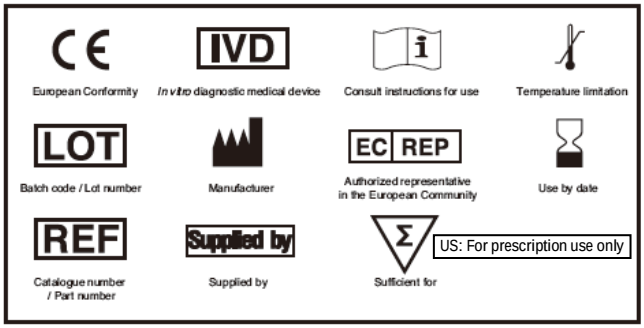
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European Conformity
IVD
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
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Manufacturer
EC REP
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Use by date
REF
Catalogue number / Part number
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Σ
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US: For prescription use only