# Boston Medical Center
# Boston MA 02118
# Department of Pathology and Laboratory Medicine

## BARC PRO 020 PAI1 ELISA

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## 1.0 **PURPOSE AND SCOPE**

1.1. The purpose of this SOP is to provide standardized instructions and guidance for measurement of Plasminogen Activator Inhibitor-1 (PAI-1) in human plasma in the Pathology and Laboratory Medicine Department of Boston Medical Center (BMC).

1.2. This procedure applies to all personnel involved in the use of this assay during the study. The goal of the SOP and associated training is to ensure consistency in measurement across samples.

## 2.0 **OVERVIEW**

2.1. PRINCIPLE OF THE ASSAY: This assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for human PAI-1 has been pre-coated onto a microplate. Standards, samples and Control are pipetted into the wells followed by a biotin-conjugated secondary antibody. An avidin-horseradish peroxidase conjugate and TMB substrate are the detection reagents and color is developed which is proportional to analyte concentration. The color development is stopped and the intensity of the color is measured. Assay quality control criteria are applied to the background, calibrator and control samples to validate the assay run. Quality control criteria are then applied to the unknown samples and data reporting guidelines are defined.

2.2. CLINICAL SIGNIFICANCE: Plasminogen Activator Inhibitor (PAI-1), also known as Serpin E1, is a member of the serpin superfamily of serine protease inhibitors. PAI-1 is the primary inhibitor of urokinase-type and tissue-type plasminogen activators (uPA and tPA), which convert plasminogen to plasmin, the primary physiologic enzyme that cleaves fibrin during fibrinolysis. The PA-plasmin system is involved in additional processes, including fibrosis, angiogenesis, wound healing, and tumor cell invasion and metastasis. The absence of PAI-1 in plasma causes abnormal bleeding, and high plasma PAI-1 levels are associated with inherited thrombophilia.

2.3. SPECIMEN REQUIREMENT: Human platelet-poor plasma (citrate, heparin or EDTA anticoagulant). A minimum of 200 microliters (200 µL) plasma is needed for each sample.

## 3.0 RESPONSIBILITY

3.1. Principal Investigator. It is the responsibility of the Principal Investigator (PI) at BMC to ensure that project personnel have been trained in accordance with this SOP, that the training is documented, and that this procedure is followed.

3.2. Project Personnel. It is the responsibility of the project lab personnel to ensure he/she has read, understands, and follows the SOP when working with blood samples and the data.

3.3. It is the responsibility of the project staff designated by the PI or BSS to ensure that all the required case report forms (CRFs) in the Comprehensive Data Resource (CDR) are completed.

3.4. Any deviation or change from this SOP, known prior to a collection, should be approved by the Leidos Technical Project Manager (TPM) and ***well documented by the site***.

3.5. *Any deviation or change that is unexpected or identified during or after a collection should be well documented by the site*. Such deviations should be submitted to the TPM along with a corrective action description for documentation.

## 4.0 **DEFINITIONS and ACRONYMS**

4.1. Acronyms- see Table 1

**Table I. Acronyms**

| **Acronym** | **Name** |
|---|---|
| PAI-1 | Plasminogen Activator Inhibitor-1 |
| CV | coefficient of variation |
| HBSS | Hank's balanced salt solution |
| ID | Identification/ Identifier |
| LLQ | lower limit of quantification |
| PBS | phosphate buffered saline |
| SD | standard deviation |
| SOP | standard operating procedure |
| UA | unanalyzable |
| ULQ | upper limit of quantification |
| | |

4.2. Assay Procedure Summary

Prepare all reagents, samples and standards as directed.

Add 50 µl of **Assay Diluent** to each well.

Add 50 µl of **Standards, Samples or Control** to each well and incubate for 2 hours at room temperature.

Aspirate and wash 4 times.

Add 200 µl of **PAI-1 Conjugate** to each well
and Incubate for 2 hours at room temperature.

Aspirate and wash 4 times.

Add 200 µl of **Substrate** to each well
and Incubate for 30 minutes at room temperature. Protect from Light

Add 50 µl of **Stop Solution**

Read Immediately at 450 nM

## 5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1. Universal Safety Precaution will be followed.

## 6.0 CRITICAL REAGENTS, MATERIALS, AND EQUIPMENT REQUIRED

6.1. Human platelet-poor plasma sample(s) handled as per SOP BARC PRO 0023 (Blood sample processing, storage, and shipping). Samples can be anticoagulated with citrate, heparin or EDTA from blood obtained in standard vacutainer collection tubes.

6.2. Human Serpin E1/PAI-1 Immunoassay kit (R&D Systems, Inc. Minneapolis, MN 55413), catalogue DSE100

**Table II. Critical Reagents**

| Reagent | Vendor | Catalog # | Storage | Notes |
|---|---|---|---|---|
| Normal human pooled plasma in 4% trisodium citrate | commercial vendor or pooled from donors | | 4-8°C, sterile | Prepare BMC Control |

| | | | | |
|---|---|---|---|---|
| Recombinant human PAI-1 | R&D Systems | 1786-PI-010 | 3 months after re-constitution, sterile, -80°C | 10 µg lyophilized |
| Human Serpin E1/PAI-1 Microplate | 893056 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal anti-PAI-1 | up to 1 month at 2-8 °C. | |
| Human Serpin E1/PAI-1 Conjugate | 893057 | 21 mL polyclonal anti-PAI-1 conjugated to horseradish peroxidase | up to 1 month at 2-8 °C. | |
| Human Serpin E1/PAI-1 Standard | 893058 | Recombinant human Serpin E1 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | up to 1 month at 2-8 °C. | |
| Assay Diluent RD1-57 | 895207 | 11 mL of a buffered protein base with preservatives. | up to 1 month at 2-8 °C. | |
| Calibrator Diluent RD5-17 | 895512 | 21 mL of a buffered protein base with preservatives. | up to 1 month at 2-8 °C. | |
| Wash Buffer Concentrate | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. | up to 1 month at 2-8 °C. | |
| Color Reagent A | 895000 | 12 mL of stabilized hydrogen peroxide. | up to 1 month at 2-8 °C. | |
| Color Reagent B | 895001 | 12 mL of stabilized chromogen (tetramethylbenzidine). | up to 1 month at 2-8 °C. | |
| Stop Solution | 895032 | 6 mL of 2 N sulfuric acid. | up to 1 month at 2-8 °C. | |
| Sodium acetate anhydrous | Sigma Aldrich | S2889-250G | Store at RT | Prepare BMC Control |
| Sodium Chloride | Sigma Aldrich | S7653-250G | Store at RT | Prepare BMC Control |

6.3. Reagent Comments

6.3.1. Seal unused wells with adhesive tape. Return to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

6.3.2. Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

6.4. Consumables- See Table III

**Table III. Consumables**

| Item | Range / Capacity | Quantity | Suggested Vendor / Catalog # |
|---|---|---|---|
| Pipet tips | 100-1000 µL | 1 box | |
| Pipet tips | 20-200 µL | 1 box | |
| Pipet tips | 0.5-10 µL | 1 box | |
| Volumetric pipette with dispenser or bulb | 5ml | at least 2 | |
| Polystyrene round bottom test tubes | 12x75mm | about 20 | |
| 1.5-mL tubes, O-ring screw cap, conical bottom, sterile | 0.5 mL | | Sarstedt 72.692.005 |
| Polypropylene tubes, sterile | 15 mL | | VWR 21008-918 |
| Polypropylene tubes, sterile | 50 mL | | VWR 21008-951 |
| Sealing tape for 96 well plates | | | Thermo Fisher 15036 |
| Disposable reagent reservoirs | | | ThermoFisher 95128095 |
| | | | |
| | | | |

6.5. Equipment—see Table IV

**Table IV. Equipment**

| Equipment | Range/Capacity | Manufacturer | Model | Serial No |
|---|---|---|---|---|
| Pipettor | 100-1000 µL | | | |
| Pipettor | 20-200 µL | | | |
| Pipettor | 0.5-10 µL | | | |
| Multichannel Pipettor | 30-300 µL | | | |
| Microplate Washer | | BioTek | ELx50 | 259186 |
| Microplate Reader | | Molecular Device | VersaMax | BNR06440 |

| | | | | |
|---|---|---|---|---|
| magnetic stirrer, stir bar | | | | |

6.6. Reagent storage and stability

6.6.1. Record the date of receipt, lot number, provided reagent concentration and expiration date for all Critical Reagents in the Batch Record (Appendix 2, Section 1).

6.6.2. All critical reagents are to be labelled with date of receipt and stored under the specified conditions for no longer that the recommended duration.

6.6.2.1. Check dates on all vials and replace any that are expired.

6.6.2.2. Storage conditions and expiration dates for all Critical Reagents are provided on the package inserts.

6.6.2.3. Do not exchange reagents from one set of qualified Critical Reagents with a set of reagents qualified separately.

6.6.2.4. Do not use any materials past expiration date.

## 7.0 . OPERATING PROCEDURE

7.1. Prior to beginning the assay, refer to the Plate Map Design and Batch Record to review all actions required for successful assay setup (Appendices 1 and 2).

7.2. Record the name and certification number of the Certified Assay Operator and the facility running the SOP in the Batch Record (Appendix 2). Include reference to 96-well plate ID, if applicable.

7.3. Plate Map and Reagent Preparation

7.3.1. Based on the number of patient samples to be analyzed, generate a Plate Map (Appendix 1) to define the location and replicates of clinical samples, control samples, and PAI-1 standards. A single patient's **batched** samples should be contained on one 96-well plate, not split over two, to ensure consistent sample handling.

**Important:** The data analyses template is based on the 96-well sample designations in the Plate Map (Appendix 1). To prevent user errors, always load the plate according to the plate map well designations.

7.3.2. Once the number of wells is known, determine the amount of reagents required for the assay using the Batch Record in Appendix 2. Once these calculations are complete, check that sufficient reagents and supplies are on hand to complete the assay.

7.3.3. Record serial numbers of equipment in the Batch Record (Appendix 2, Section 5).

7.4. **Pre-Assay Reagent Preparation**

7.4.1. Preparation of BMC PAI-1 Control

7.4.2. Re-suspend Recombinant PAI-1 in 1 mL of 50 mM sodium acetate and 100 mM NaCl, pH 5.5. Final concentration in vial is 10 µg/mL.

7.4.2.1. The buffer solution is made by adding 410.2 mg of sodium acetate and 584.4 mg sodium chloride (NaCl) to 80 mL distilled or deionized water in a glass beaker or other suitably sized vessel. Solubilize by mixing with a stir bar and magnetic stirrer. Adjust the pH to 5.5 with hydrochloric acid (HCl). Adjust to a final volume of 100 mL with distilled or deionized water. Store at 2-8$^{o}$C until needed. Allow to come to room temperature before solubilization of lyophilized recombinant PAI-1.

7.4.3. Label 50 mL conical “PAI-1 Control.” Add 20 µL of PAI-1 stock solution to 40 mL of pooled human citrated plasma (commercial vendor or donors). Store remaining stock solution in 20 µL aliquots at -80$^{o}$C (about 49). Store remaining pooled plasma in 200 µL aliquots at -80$^{o}$C.

7.4.4. Aliquot PAI-1 Control in 200 µL aliquots (about 200) and store at -80$^{o}$C for future use.

7.5. **Reagent Preparation on Assay Day:** All reagents should be at room temperature prior to assay

7.5.1. Preparation of Wash buffer

7.5.1.1. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of wash buffer concentrate to deionized or distilled water to prepare 500 mL Wash Buffer.

7.5.2. Preparation of PAI-1 Conjugate

7.5.2.1. Tap the vial of Conjugate to dislodge any liquid from the cap. Ensure contents are mixed by gentle swirling. The PAI-1 Conjugate is ready for use in the assay and requires no further dilution.

7.5.3. Preparation of Substrate Solution

7.5.3.1. Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. Record mixing time and time of addition to the wells in the batch record (Appendix 2, Section 3C,D)

7.6. **Preparation of Standards (for triplicates on each plate)**

7.6.1.1. Reconstitute the lyophilized human PAI-1 standard with deionized or distilled water. Refer to the kit vial label for reconstitution volume. This makes a stock solution of 200 ng/mL PAI-1. Mix by gentle swirling to ensure complete reconstitution. Let sit for at least 15 minutes at room temperature with occasional mixing prior to making dilutions. **Vigorous agitation and foaming should be avoided**.

7.6.1.2. Label eight 1.5-mL tubes, numbered 1 through 8, for the PAI-1 standards. Prepare the PAI-1 standards by serial dilution with Calibrator Diluent RD5-17 with final concentrations of 20 to 0 ng/mL. Vortex briefly after each addition. Change pipette tips between each transfer. See Table V for dilutions. Serially diluted Standards will be added directly to the 96-well plate with no further dilution.

**Table V. Preparation of Standards**

| Standard # | Concentration (ng/mL) | Volume Calibrator Diluent RD5-17 (µL) | Volume PAI-1 (µL) | Final concentration in assay (ng/mL) |
|---|---|---|---|---|
| 1 | 20 | 450 | 50µL stock | 10 |
| 2 | 10 | 200 | 200 µL #1 | 5 |
| 3 | 5 | 200 | 200 µL #2 | 2.5 |
| 4 | 2.5 | 200 | 200 µL #3 | 1.25 |
| 5 | 1.25 | 200 | 200 µL #4 | 0.625 |
| 6 | 0.625 | 200 | 200 µL #5 | 0.313 |
| 7 | 0.313 | 200 | 200 µL #6 | 0.156 |
| 8 | 0 | 200 | 0 | 0 |
| (Volume, µL) | | (1,850) | | |

7.7. Preparation of Unknowns (plasma samples)

7.7.1.1. No dilution of plasma samples is required.

7.8. **ASSAY PROCEDURE**

7.8.1. Prepare all reagents, working standards, control and samples as directed in section 7.5.

7.8.2. Remove excess microplate strips from the plate frame, reseal with adhesive tape and return them to the foil pouch containing the desiccant pack. Reseal the pack

7.8.3. Add 50 µL of Assay Diluent RD1-57 to each well.

7.8.4. Add 50 µL of Standard, control, or sample per well as shown in the Plate Map (Appendix 1). Cover with the adhesive strip.

**Important: Ensure that addition to the plate is uninterrupted and completed within 15 minutes.**

7.8.5. Incubate for 2 hours at room temperature on the shaker. Record the date, starting time, and incubation temperature in the batch record (Appendix 2, Section 3A)

7.8.6. Wash

7.8.6.1. Following incubation with PAI-1 Standards and Samples, aspirate each well and wash, repeating the process three times (total four washes).

7.8.6.2. Wash by using an automatic plate washer (BioTek ELx50).

7.8.6.3. Complete removal of liquid at each step is essential to good performance.

7.8.6.4. After the last wash, invert the plate and tap it against clean paper towels to remove residual buffer.

7.8.6.5. Proceed immediately to the next step; do not allow the plate to dry out.

7.8.6.6. For the BioTek Microplate Washer, the settings are:

| METHOD | ELx405 Select | ELx405 |
|---|---|---|
| Number of Cycles: | 4 | 4 |
| Soak/Shake: | Yes | Yes |
| Soak Time: | 5 sec | 5 sec |
| Dispense Volume: | 400 µL/well | 400 µL/well |

7.8.7. Add 200 µL Human PAI-1 Conjugate to each well using a multichannel pipettor. Cover with a new adhesive strip.

7.8.8. Incubate for 2 hours at room temperature. Record the date, starting time, and incubation temperature in the batch record (Appendix 2, Section 3B).

7.8.9. Wash: repeat the aspiration/wash in step 7.6.6.

7.8.10. Add 200 µL of Substrate Solution to each well using a multichannel pipettor.

7.8.11. Incubate for 30 minutes at room temperature and protect from light; start incubation after addition of substrate to the last column. Record the date, starting time, and incubation temperature in the batch record (Appendix 2, Section 3D).

7.8.12. Add 50 µL Stop Solution to each well using a multichannel pipettor. The Stop Solution should be added to the wells in the same order as the Substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7.8.13. Determine the Optical Density

7.8.13.1. Determine the optical density (OD) of each well within 30 minutes, using a microplate reader set to 450 nm with wavelength correction set to 540 or 570 nm to correct for imperfections in the plate. If wavelength correction is not available, subtract readings at 650 nm from the readings at 450 nm. Readings made directly at 450 nm without correction may be higher and less accurate.

7.8.13.2. Save the resulting readings to a secure computer; recommended to label the file with the date and a unique assay identifier (Plate ID): PAI-1 ELISA MM/DD/YEAR PLATEX format (e.g., PAI-1 ELISA 03062017 PLATE1). Record the file name in the Batch Record (Appendix 2, Section 4B). Print a paper copy of the raw data for inclusion with the Batch Record.

7.9. Review and finalize the Batch Records (Appendix 2) and obtain required signature. Document ANY and ALL deviations from this SOP in the Batch Record (Appendix 2, Section 7-8).

## 8.0 DATA ANALYSIS

### 8.1. **PRINCIPLE:**

8.1.1. Optical density data is converted to antigen (PAI-1) concentration with a computer program, SoftMax Pro. Acceptable results are obtained with computer programs using a standardized curve-fitting four parameter logistic method, or a logistic/log regression analysis.

8.1.2. The protocol calls for a “PAI-1 ELISA Analysis”, which tells the calculation-program the location of samples, standards, QC, the initial dilution and serial dilutions. Wells designated as S Diluent Only in the Plate Map (Appendix 1) should be labeled as “blank wells” in the template. The program should subtract the average OD of the “blank wells” from the OD of other wells.

8.1.3. The soluble PAI-1 concentration for each sample is found by calculating the mean of the sample duplicate and multiplying by the dilution factor (2).

### 8.2. **DATA INSPECTION RULES**

8.2.1. Blanks: the signal of blank wells should be less than 0.2 units for all assay plates. If any blank wells are >0.2, the assay should be examined for inappropriate results and should be re-assayed if no apparent causes are found.

8.2.2. Triplicates: If the coefficient of variation (CV) of triplicate wells is >15% and two wells have a CV of ≤ 10%, then the outlier well value can be excluded from the calculation. This has to be documented in Appendix 2, section 7. If > 1 outlier well is observed, the assay should be examined for cause and re-assayed if no apparent causes are found.

8.2.3. Standards: The slope of the linear portion of the reference standard curve (e.g., OD 0.1 to 2.0) should be near 1.0 (0.9 – 1.1) when the log of the OD signal is graphed against the log of the standard concentration.

8.2.4. Sensitivity: Calculate the lower detection limit for the assay and confirm that the detection limit is within in the established range.

8.2.5. Quality Control: The Control sample (from kit or prepared from commercial reagents) must be within the established range (mean ± 3 SD), or the plate is rejected and samples are re-analyzed.

8.2.6. If a sample has OD readings greater than 2.0 in the assay at the highest dilution used in the assay, the sample should be re-assayed after additional dilution.

8.2.6.1. If an unknown value is high and is diluted more than that defined in the assay procedure, then new controls should be made with normal human pooled plasma using the same dilution factor to replicate the amount of plasma in all the samples.

8.2.7. If the PAI-1 concentration of the sample was calculated by averaging the data from multiple dilutions and the CV of the concentration exceeds 30%, then the data should be examined for inappropriate results and should be re-assayed if no apparent causes are found.

8.2.8. If the lower limit of detection is equal to or less than the established value and a sample has undetectable PAI-1 concentration, report one half of the established assay lower limit as the concentration for the sample. If the lower limit of detection is more than the established value and a sample has undetectable PAI-1 concentration, do not report the result for the sample and reanalyze the sample.

8.3. **DATA ANALYSIS:** Most software analysis packages, including SoftMax Pro, will perform curve fitting and data analysis to obtain concentrations.

8.3.1. Obtain average OD of serially diluted Standards and each sample wells.
8.3.2. For each antigen concentration, obtain the 'signal' by subtracting the average OD value of the background wells from the average OD value of the corresponding wells that contain standards or unknown.
8.3.3. Plot the background corrected signal values on the Y-axis and the logarithm of PAI-1 standard concentration on the X-axis to obtain the standard curve.
8.3.4. Obtain unknown concentrations from the standard curve. Multiply by any dilution to obtain the final PAI-1 concentration.

## 9.0 REFERENCE

9.1. R&D User's Guide for Human SE1/PAI-1 Immunoassay.

9.2. National Clinical Target Validation Laboratory, Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc by Frederick National Laboratory for Cancer Research.

## 10.0 ATTACHMENTS

| INITIATION/REVISION HISTORY | | | |
|---|---|---|---|
| **REV #** | **DESCRIPTION OF CHANGE** | **AUTHOR** | **EFFECTIVE DATE** |
| 1.0 | Draft | John Kim | |
| 1.1 | Draft | DSK, MT | 5/15/2017 |
| 2.0 | Approved draft | DSK, MT | 7/17/2017 |
| 2.1 | Minor Clarifications, typos, formatting | BET,DSK,ERD,MPT | 08/01/2018 |

**APPENDIX 1: PLATE MAP DESIGN** Patient samples from Module I and II may be assayed on the same plate (same design), but the pre-analytic variable grouping for each patient must be included on the same plate.

- When only 1 or 2 patient samples (S) are run, the Plate Map Design can be adjusted, so long as triplicate wells are used for samples, standards and controls.
- Blank wells are loaded with Reagent Diluent only (no sample).
- Document the sample/patient IDs and other pertinent information in the Sample Calculation Table in the Batch Record (Appendix 2).

**A1.1 Module I Plate Design (Time to Centrifuge): Room Temperature Assay**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | STDS | | S1T1 | | | S9T4 | | | S17T2 | | |
| B | | | | S2T2 | | | S10T1 | | | S18T4 | | |
| C | | | | S3T4 | | | S11T2 | | | S19T1 | | |
| D | | | | S4T1 | | | S12T4 | | | S20T2 | | |
| E | | | | S5T2 | | | S13T1 | | | S21T4 | | |
| F | | | | S6T4 | | | S14T2 | | | | | |
| G | CTL | CTL | CTL | S7T1 | | | S15T4 | | | | | |
| H | BMC | BMC | BMC | S8T2 | | | S16T1 | | | Blank | | |

**A1.2 Module II Plate Design (Freeze-Thaw Cycles): Room Temperature Assay**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | STDS | | S1C1 | | | S9C3 | | | S17C2 | | |
| B | | | | S2C2 | | | S10C1 | | | S18C3 | | |
| C | | | | S3C3 | | | S11C2 | | | S19C1 | | |
| D | | | | S4C1 | | | S12C3 | | | S20C2 | | |
| E | | | | S5C2 | | | S13C1 | | | S21C3 | | |
| F | | | | S6C3 | | | S14C2 | | | | | |
| G | CTL | CTL | CTL | S7C1 | | | S15C3 | | | | | |
| H | BMC | BMC | BMC | S8C2 | | | S16C1 | | | Blank | | |

## APPENDIX 2: BATCH RECORD

**NOTE:** Record times using **military** time (24-h designation); for example, specify 16:15 to indicate 4:15 PM.

Certified Assay Operator: _____ Certification Number:_____

Facility/Laboratory Running SOP: _____

Clinical Protocol Number: _____

Date Immunoassay Run: _____

Plate ID (optional): _____

### 1. Critical Reagents

The critical reagents are listed below; complete the table as designated. Be sure the lot numbers on each of the reagents match those cited in the product insert accompanying the reagents. Reagents from one kit **should not** be exchanged with reagents from another.

| Reagent Name | Date Received | Lot No | Provided Reagent | Recommended Dilution/Conc for working solution | Exp Date |
|---|---|---|---|---|---|
| Human PAI-1 Microplate | / / | | N/A | N/A | / / |
| Human PAI-1 Standards | / / | | Lyophilized powder | | / / |
| Human PAI-1 Conjugate | / / | | 21 ml/vial | | / / |
| Assay Diluent RD1-57 | / / | | 11 mL/vial | | / / |
| Calibrator Diluent RD5-17 | / / | | 21 mL/vial | | / / |
| Wash Buffer Concentrate | / / | | 21mL | | / / |
| Color Reagent A | / / | | 12 mL/vial | | / / |
| Color Reagent B | / / | | 12 mL/vial | | / / |
| Stop Solution | / / | | 6 mL/vial | | / / |

**2. Unknown Samples**

| Sample No | Sample/Patient ID | Module/PAV | Dilution (X) | | |
|---|---|---|---|---|---|
| **S Ex** | TCP_0001 | I / T2 | 2 | | |
| **S1** | | | | | |
| **S2** | | | | | |
| **S3** | | | | | |
| **S4** | | | | | |
| **S5** | | | | | |
| **S6** | | | | | |
| **S7** | | | | | |
| **S8** | | | | | |
| **S9** | | | | | |
| **S10** | | | | | |
| **S11** | | | | | |
| **S12** | | | | | |
| **S13** | | | | | |
| **S14** | | | | | |
| **S15** | | | | | |
| **S16** | | | | | |
| **S17** | | | | | |
| **S18** | | | | | |
| **S19** | | | | | |
| **S20** | | | | | |
| **S21** | | | | | |

**3.0 Plate Incubation**

a. Add clinical samples, controls, and PAI-1 standards to the 96-well plate, cover plate, and incubate at room temperature for 2 hours on the shaker.

| Date | Start | Stop | Incubation Temp (°C) |
|---|---|---|---|
| / / | : | : | |

b. Add Human PAI-1 Conjugate to the 96-well plate, cover plate, and incubate at room temperature for 2 hours on the shaker.

| Date | Start | Stop | Incubation Temp (°C) |
|---|---|---|---|
| / / | : | : | |

c. Mix Substrate solution

| Date | Start | Stop | Incubation Temp (°C) |
|---|---|---|---|
| / / | : | : | |

d. Add Substrate Solution to the 96-well plate, and incubate at room temperature for 30 minutes.

| Date | Start | Stop | Incubation Temp (°C) |
|---|---|---|---|
| / / | : | : | |

**4. Software:**

a. SoftMax Pro Version:_____

b. Name of original SoftMax Pro data file: _____

5. **Equipment:** Standard equipment is listed below. Check if used for the biomarker assay. If different equipment was used, document in Appendix 2, Section 7

| Check if used | Equipment | Manufacturer | Model | Serial No |
|---|---|---|---|---|
| | Microplate Washer | BioTek | | ELx50 |
| | Microplate Reader | Molecular Devices | | VersaMax |
| | Spectrofluorometer | Molecular Devices | Gemini XPS | XPS05453 |
| | Refrigerator (2-8$^{o}$C) | | | |
| | Freezer (-80$^{o}$C) | | | |

**6. Plate Map QC**

a. Name of saved Human PAI-1 Excel data analysis workbook
_____

b. Plate Map Set Up QC
( ) Recommended human PAI-1 Plate Map used.
( ) Alternative plate map used; cells copy and pasted individually to the Plate Layout QC worksheet.
Reason: _____

**7. Notes, including any deviations from the SOP:**
If assay fails QC, state the specific reason for assay failure and notify the Laboratory Director/Supervisor.

**8. Laboratory Director/Supervisor Review of Batch Record**
Laboratory Director/Supervisor: _____ (Print)

_____ (Sign)

**9.** Date: _____

**APPENDIX 3: Work Process Flow**

OVERVIEW OF IMMUNOASSAY SAMPLE PROCESSING

**BARC PRO 012**:

Thrombosis in Cancer Patients: Blood sample Collection SOP

- Properly collect blood at all BSSs for the the Thrombosis in Cancer Patients Pre-Analytical Factors (PAF) study.
- Immediately invert the tube slowly and gently.
- Transport to blood processing laboratory.

**BARC PRO 023**:

Thrombosis in Cancer Patients: Blood sample Processing, Storage and Shipping

- Instruction to biospecimen source sites for blood sample processing, storage and shipping.
- Blood will be processed for the preparation of blood derivatives from all study donors for downstream marker analyses.
- Collected Plasma (200 ul) will be aliquoted to a pre-labeled cryovial for PAI-1 ELISA.

**BARC PRO 020**:

Thrombosis in Cancer Patients: Immunoassay of Human SE1/PAI-1in blood sample

- Perform ELISA with clinical samples, controls and PAI-1 standards.
- Using Microplate reader, determine relative signal of all samples