Boston Medical Center
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Department of Pathology and Laboratory Medicine

BARC PRO 016 pSelectin SOP

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Version	Status	Type	Date Added	Date Effective	Date Retired
2.1	Approved and Current	Minor revision	8/20/2018	8/20/2018	Indefinite
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BOSTON MEDICAL CENTER	Thrombosis in Cancer Patients Soluble P Selectin 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 human Soluble P-Selectin (sPS) in human plasma in the Pathology and Laboratory Medicine Department of Boston Medical Center.

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 P-selectin has been pre-coated onto a microplate. Standards, samples and Control are pipetted into the wells together with a polyclonal antibody specific for human P-Selectin which has been conjugated to horseradish peroxidase. Following a wash to remove any unbound conjugated antibody, a substrate is added and color is developed which is proportional to analyte concentration. 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: P-Selectin, also known as GMP-140, LECAM-3, PADGEM, and CD62P, is a cell surface glycoprotein that plays a critical role in the migration of lymphocytes into tissues. It is found constitutively in a pre-formed state in the Weibel-Palade bodies of endothelial cells and in the alpha granules of platelets. This stored P-Selectin is mobilized to the cell surface within minutes in response to a variety of inflammatory or thrombogenic agents. The mobilized P-Selectin is apparently present on the cell surface for only a few minutes after which it is recycled to intracellular compartments. P-Selectin consists of an NH_2-terminal lectin type C domain, an EGF-like domain, nine complement control domains, a transmembrane domain, and a short cytoplasmic domain. The extracellular domains can be cleaved and released into the circulation for detection as a biomarker of endothelial activation.

2.3. SPECIMEN REQUIREMENT: Human platelet-poor plasma (citrate, heparin, or EDTA anticoagulant). A minimum of twenty microliters (20 µl) plasma is needed for each sample. Samples require a 20-fold dilution.

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 Biospecimen Source Site (BSS) to ensure that all the required case report forms (CRFs) in the Comprehensive Data Resource (CDR) are completed.

3.4. Any planned deviation or change from this SOP, known prior to a collection, should be approved by the Biospecimen Research Group – Quality Management (BRG-QM) and Leidos Technical Project Manager (TPM) and ***well-documented by the site***.

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

4.0 **DEFINITIONS and ACRONYMS**

4.1. Acronyms

Table I. Acronyms

Acronym	**Name**
sPS	Soluble P-Selectin
ELISA	Enzyme-Linked ImmunoSorbent Assay
HRP	Horseradish Peroxidase
HBSS	Hank's Balanced Salt Solution
CV	coefficient of variation
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: takes about 1.5 hours to complete

Prepare all reagents, samples and standards as directed.

Add 100 µl of **Sample, Standard, Control** to each well.

Add 100 µl of diluted PS **Conjugate** to each well
and Incubate for 1 hour at room temperature.

Aspirate and wash 3 times.

Add 100 µl of **Substrate** to each well
and Incubate for 15 minutes at room temperature

Add 100 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. Critical Reagents- see Table II

6.2.1. Human P-Selectin/CD62P Immunoassay kit (R&D System, Inc. Minneapolis, MN 55413, USA), Catalog number BBE6. Store up to 1 month (from the date kit was received) or shorter if suggested by Manufacturer.

6.2.2. Recombinant Human P-Selectin/CD62P Protein, CF, 50 µg for sP-selectin BMC control. (R&D System, Inc. Minneapolis, MN 55413, USA), Catalog number ADP3-050. Store stock at -80°C after reconstitution

Table II. Critical Reagents

Reagent	**Vendor**	**Catalog #**	**Storage**	**Notes**

Coated microplates	Human P-Selectin/CD62P Immunoassay kit (R&D System)	890272	up to 1 month at 2-8°C	12 strips of 8 wells
Lyophilized recombinant P-selectin Standards	6 vials, 1 vial of each level	890274-890279	stored up to 1 month at ≤ -20°C	Concentrations shown on vial labels
P-selectin control	1 vial	890280	stored up to 1 month at ≤ -20 °C	Lyophilized human serum containing P-Selectin
P-selectin conjugate concentrate	1 vial	890273	stored up to 1 month at 2-8°C	0.3mL/vial polyclonal anti-human P-selectin conjugated to HRP
Sample diluent	2 vials	895173	stored up to 1 month at 2-8°C	20mL/bottle with blue dye
Conjugate diluent	1 vial	895172	stored up to 1 month at 2-8°C	11 mL/vial with red dye and preservatives.
Wash buffer concentrate	1 vial	895003	stored up to 1 month at 2-8°C	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. May turn yellow over time.
Substrate	1 vial	895002	stored up to 1 month at 2-8°C	11 mL/vial of stabilized substrate solution (tetramethylbenzidine).
Stop solution	1 vial	895004	stored up to 1 month at 2-8°C	11 mL/vial of 1N hydrochloric acid.
Plate sealers	8 strips	n/a		adhesive strips
Recombinant Human sP-selectin	R&D Systems, Inc.	ADP3-050	Store stock at -80°C after reconstitution	1 vial, lyophilized 50µg to be reconstituted to 1mg/mL
Normal human	Sigma-Aldrich	P9523-5ML	4-8°C, sterile	Prepare BMC Control

pooled plasma in 4% trisodium citrate				
Hank's balanced salt solution (HBSS)	ThermoFisher Scientific	14025-092	Keep stock solution at room temp (~25°C)	Store in sterile 10mL aliquots at -20°C. Use once, then discard.

6.3. Reagent Comments

6.3.1. Manufacturer confirmed unopened reagents are stable at 2-8°C until expiration date. Opened reagents are stable at 2-8°C for up to 1 month from opening.
6.3.2. Cover unused wells with adhesive plate sealer and return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.
6.3.3. The concentration of the ELISA kit control should fall within the range specified on the vial label if the assay is valid.
6.3.4. Sample diluent is a buffered protein base with blue dye and preservatives.
6.3.5. Wash buffer concentrate is 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time (*BMC confirmed via inquiry to Manufacturer's Tech support that color change is normal and does not indicate bacterial contamination).

6.4. Consumables: see Table III.

Table III. Consumables

Item	Range / Capacity	Quantity	Suggested Vendor / Catalog #
Pipet tips	200-1000 µL	1 box	-
Pipet tips	50-200 µL	1 box	-
Pipet tips	2-20 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	1.5 mL	-	Sarstedt 72.692.005
Polypropylene conical tubes, sterile	15 mL	-	VWR 21008-918
Polypropylene conical 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	-	-	-
Ice Bucket	-	-	-	-
Microplate Washer	-	BioTek	ELx50	259186
Microplate Reader	-	Molecular Devices	VersaMax	BNR06440
Refrigerator	2°C to 8°C	-	-	-

6.6. **Reagent storage and stability**

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

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

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

6.6.3.1. Check dates on all vials and replace any that are ready to expire and/or expired.
6.6.3.2. Storage conditions and expiration dates for all Critical Reagents are provided on the package insert.
6.6.3.3. Do not exchange reagents from one set of qualified Critical Reagents with a set of reagents qualified separately.
6.6.3.4. Do not use kit 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 P-Selectin 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 5B). Prepare the Wash Buffer and Sample Diluent as outlined in the Batch Record (Appendix 2, Section 2B).

7.4. **Pre-Assay Reagent Preparation**

7.4.1. Prepare BMC Control for aliquot storage

7.4.1.1. Reconstitute sP-selectin protein to 1mg/mL with deionized water (stock solution). Allow to sit at room temperature for ~20 minutes to ensure complete solubilization.

7.4.1.2. Label 15mL conical "P-Selectin Working Solution." Add 5uL of sP-selectin stock solution to 495 µL of deionized water for a 10 µg/mL working solution. Aliquot remaining sP-selectin stock in 10uL aliquots.

7.4.1.3. Pipette 57.0 mL HBSS into a sterile plastic container. Label "sP-selectin BMC Control"

7.4.1.4. Pipette 3 mL normal pooled plasma (citrated) to the tube. Swirl briefly to mix. Document lot # of pooled plasma.

7.4.1.5. Pipette 150uL sP-Selectin working solution into container containing plasma and buffer. Swirl briefly to mix. Exogenous sP-selectin concentration is 25 ng/mL.

7.4.1.6. Make 400uL aliquots in screw cap tubes with O-ring. This will provide about 150 aliquots. Label and put in -80°C to freeze rapidly.

7.4.1.7. For remainder of normal human pooled plasma, make 100 µL aliquots (about 20) in screw cap tubes with O-ring. Label and put in -80°C to freeze rapidly.

7.4.1.8. Store frozen at -80°C. Controls are used once and excess is discarded.

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

7.5.1. Preparing P-Selectin Conjugate

7.5.1.1. Tap the vial of Conjugate Concentrate to dislodge any liquid from the cap. Ensure contents are mixed with a suitable pipette.

7.5.1.2. Transfer 250 µL of the Conjugate Concentrate into the bottle of Conjugate Diluent. Mix by gentle inversion and swirling. **Vigorous agitation and foaming should be avoided.** The P-Selectin Conjugate is now ready for use in the assay and requires no further dilution. Let warm to room temperature before use.

7.5.2. Preparation of wash buffer:

7.5.2.1. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

7.5.2.2. Add 20 mL of Wash Buffer Concentrate to 480 ml of deionized water to prepare 500 mL of Wash Buffer. Buffer should be room temperature before use.

7.5.3. Remove substrate and stop solution from refrigerator. Tap to dislodge any liquid from vial caps. Ensure contents are mixed. Let warm to room temperature before use.

7.5.4. Preparation of P-Selectin Standards (for triplicate on each plate)

7.5.4.1. Retrieve each vial of sPS Standard from the freezer. Reconstitute Standards immediately before use with 1.0 ml of deionized water. Allow vials to sit at room temperature for at least 10 minutes and mix by gentle inversion and swirling until all contents are completely dissolved. **Vigorous agitation and foaming should be avoided**. The standards are now ready for use in the assay and require no further dilution.

7.5.4.2. Standards will be added directly to the 96-well plate with no further dilution.

7.5.4.3. Only make enough Standards for the assay and discard any excess stock or diluted standards.

7.5.5. Preparation of P-selectin Control supplied in ELISA kit

7.5.5.1. Thaw and reconstitute P-selectin control immediately before use with 500 uL of distilled or deionized water. Allow the control to sit at room temperature for at least 10 minutes. Mix by gentle inversion and swirling

7.5.5.2. Dilute 20-fold in sample diluent prior to adding to plate wells by mixing 20 µL P-selectin control + 380 µL Sample Diluent.

7.5.6. Preparation of Unknown (plasma samples)

7.5.6.1. Thaw plasma samples rapidly at 37^{o}C, then place all plasma samples to be assayed on ice until ready to use.

7.5.6.2. All plasma samples are diluted 20-fold into Sample Diluent by mixing 20 µL of plasma + 380 µL of Sample Diluent.

7.6. **ASSAY PROCEDURE**

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

7.6.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

7.6.3. Add 100 µL Standard, Control, BMC Control, sample or sample diluent (blank wells) as shown in the Plate Map (Appendix 1). CTL is the control supplied with the ELISA kit. BMC is the control made with commercial human plasma and recombinant sPS.

7.6.4. Add 100 uL diluted P-selectin conjugate to each well.

7.6.5. Mix by tapping plate gently and cover the plate with an adhesive seal.

7.6.6. Incubate for 1 hour at room temperature. Record the date, start time, and incubation temperature in the Batch Record (Appendix 2, Section 3a).

7.6.7. Wash

7.6.7.1. Following incubation, aspirate the plate using a plate washer (for the BioTek Plate Washer, use the *ELISA 1* program). Immediately wash the plate 3 times with 300 µL Wash Buffer, aspirating the plate between each wash and being sure no residual liquid remains. Addition of a 30 second soak period after dispense of the wash buffer is recommended by the manufacturer. Rotating the plate 180 degrees between wash steps may improve assay precision.

7.6.7.2. After the wash, tap the plate on paper towels to remove residual buffer. Proceed immediately to the next step; do not allow the plate to dry out.

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

METHOD	ELx405 Select	ELx405
Number of Cycles:	3	3
Soak/Shake:	Yes	Yes
Soak Time:	5 sec	5 sec
Dispense Volume:	400 µL/well	400 µL/well

7.6.8. Adding substrate

7.6.8.1. Add 100 µL of Substrate to each well using a multichannel pipettor. Cover the plate with a new plate sealer and incubate at room temperature for 15 minutes.

7.6.8.2. Record the date, starting time, and incubation temperature in the Batch Record (Appendix 2, Section 3b).

7.6.9. Adding Stop Solution

7.6.9.1. Add 100 µL of 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.

7.6.10. Determine Optical Density

7.6.10.1. Determine the optical density of each wells within 30 minutes, using a microplate reader set to 450 nm with wavelength correction set to 620 nm to correct 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.

7.6.10.2. Save the resulting readings to a secure computer; recommended to label the file with the date and a unique assay identifier (Plate ID): sPS ELISA MM/DD/YEAR PLATEX format (e.g., SPS 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.

7.7. 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).

8.0 DATA ANALYSIS

8.1. **PRINCIPLE:**

8.1.1. Optical density data is converted to antigen (sPS) 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 “sPS 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 P-Selectin concentration for each sample and control is found by calculating the mean of the sample replicates and multiplying by the dilution factor (20).

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 slop 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: Each 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 an average OD average greater than that of the highest standard, 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 sPS 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 sPS 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 PS 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, Controls and each sample well.

8.3.2. For each sPS 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 sPS 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 sPS concentration.

9.0 REFERENCE

9.1. R&D User's Guide for Human P-Selectin/CD62P 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	John Kim, DSK	March 15, 2017
1.2	Draft	DSK, MT	Apr 7, 2017
1.3	Draft	DSK, MT	May 15, 2017
2.0	Approved draft	DSK, MT	7/17/2017
2.1	Minor Clarifications, typos, formatting	BET,DSK,ERD,MPT	8/1/2018

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/ or created	Lot No	Provided Reagent	Recommended Dilution/Conc for working solution	Exp Date
P-Selectin Microplate	/ /		N/A	N/A	/ /
P-Selectin Standards	/ /		Lyophilized powder		/ /
P-Selectin Control	/ /		Lyophilized powder		/ /
BMC P-Selectin Control	/ /		0.4mL/vial		/ /
P-Selectin Conjugate	/ /		0.3mL/vial		/ /
Sample Diluent	/ /		20mL/bottle		/ /
Conjugate Diluent	/ /		11mL/vial		/ /
Wash Buffer Concentrate	/ /		21mL/vial		/ /
Substrate	/ /		11mL/vial		/ /
Stop Solution			11mL/vial		/ /

2. Unknown Samples

Sample No	Sample/Patient ID	Module/PAV	Dilution (X)		
S Ex	TCP_0001	I / T2	20		
S1					
S2					
S3					
S4					
S5					
S6					
S7					
S8					
S9					
S10					
S11					
S12					
S13					
S14					
S15					
S16					
S17					
S18					
S19					
S20					
S21					

3. Plate Incubation

a. Add clinical samples, P-Selectin controls, and P-Selectin standards, P-Selectin Conjugate to the 96-well plate, cover plate, and incubate at room temperature for 1 h.

Date	Start	Stop	Incubation Temp (°C)

/ /	:	:	

b. Add Substrate to the 96-well plate, cover plate, and incubate at room temperature for 15 minutes.

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

4. Software:

a. SoftMax Pro Version:_____

b. Name of original SoftMax 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 sPS Excel data analysis workbook

_____

b. Plate Map Set Up QC

( ) Recommended sPS 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)

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 (TCP) 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 will be aliquoted to a pre-labeled cryovial for sP-Selectin ELISA.

BARC PRO 016: Thrombosis in Cancer Patients: Immunoassay of soluble P-Selectin in blood sample	• Perform ELISA with clinical samples, P-Selectin standards, and P-Selectin Controls • Using Versa Max Microplate reader, determine relative signal of all samples