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

BARC PRO 019 Myeloperoxidase SOP

Copy of version 2.1 (approved and current)

Last Approval or Periodic Review Completed 8/20/2018

Next Periodic Review Needed On or Before 8/20/2019

Effective Date 8/20/2018

Controlled Copy of a Manual ID 15099

Location TCP SOP SharePoint

Organization Boston Medical Center

Description
Not final format

Comments for version 2.0 (last major revision)
Major Format Changes including plate layouts

Comments for version 2.1 (this revision)
Typos and clarifications

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Periodic review	Laboratory Director Review	8/20/2018	2.1	Chris Andry, PhD Chris Andry	
Approval	QA Review	8/20/2018	2.1	ERDuffy Elizabeth Duffy	
Approval	Administrative Director	7/19/2017	2.0	Chris Andry, PhD Chris Andry	
Approval	Lab Director	3/23/2017	1.0	Chris Andry	Recorded when document uploaded to MediaLab
Periodic review	Designated Reviewer	3/23/2017	1.0	Chris Andry	Recorded when document uploaded to MediaLab

Approvals and periodic reviews that occured before this document was added to the MediaLab Document Control system may not be listed.

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
2.1	Approved and Current	Minor revision	8/20/2018	8/20/2018	Indefinite
2.0	Retired	Major revision	7/14/2017	7/19/2017	8/20/2018
1.0	Retired	First version in Document Control	3/23/2017	3/23/2017	7/19/2017

BOSTON MEDICAL CENTER	Thrombosis in Cancer Patients Myeloperoxidase assay		
BARC PRO 019	Version 2.1	Effective Date: 08/01/2018	Page **1** of **20**

1.0 PURPOSE AND SCOPE

1.1. The purpose of this SOP is to provide standardized instructions and guidance for measurement of Myeloperoxidase (MPO) 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:
For ELISAs: This assay employs the quantitative sandwich immunoassay technique. This assay employs the quantitative sandwich immunoassay technique. A monoclonal antibody specific for human Myeloperoxidase has been pre-coated onto a microplate. Standards, samples and control are pipetted into the wells followed by a secondary polyclonal antibody specific for MPO conjugated to horseradish peroxidase. The enzyme substrate is added 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.

CLINICAL SIGNIFICANCE: Myeloperoxidase (MPO) is a heme-containing enzyme belonging to the XPO subfamily of peroxidases. It is an abundant neutrophil and monocyte glycoprotein that catalyzed the hydrogen peroxide dependent formation of hypochlorus acid (HOCl) and other reactive species. Neutrophil MPO is stored in cytoplasmic azurophilic granules. Upon cellular activation and degranulation, MPO is delivered into phagosomes where it is required for the killing of phagocytosed bacteria. Activated neutrophils also release granule contents extracelluarly. Elevated plasma MPO levels have been associated with a variety of clinical conditions including systemic inflammation, eclampsia, risk of cardiovascular events, vascular endothelial dysfunction, severity of multiple sclerosis, and prospective mortality and oxidative stress during hemodialysis.

2.2. SPECIMEN REQUIREMENT: Human platelet-poor plasma (citrate, heparin or EDTA anticoagulant). A minimum of 20 microliters (20 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 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- see Table I.

Table I. Acronyms

Acronym	**Name**
MPO	Myeloperoxidase
CV	coefficient of variation
HBSS	Hank's balanced salt solution
ID	Identification/ Identifier
LLQ	lower limit of quantification
OD	optical density
SD	standard deviation
SOP	standard operating procedure
UA	unanalyzable
ULQ	upper limit of quantification

4.2 Assay Procedure Summary: About 5 hours

Prepare all reagents, samples and standards.

Add 100 µL assay diluent to wells, then 50 µl of Sample, Standard, Control or Blank.

Incubate for 2 hours at room temperature.

Wash, Add 200 uL MPO conjugate Antibody. Incubate for 2 hours at room temperature.

Wash, add Substrate. Incubate for 30 minutes

Add Stop solution. Read OD at 450nM

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- Human Myeloperoxidase Immunoassay kit (R&D Systems, Inc. Minneapolis, MN 55413), catalogue #DMYE00B . See Table II

Table II. Critical Reagents

Reagent	Vendor	Catalog #	Storage	Notes
Hank's balanced salt solution (HBSS)	ThermoFisher Scientific	14025-092	keep stock solution bottles at room temp (~25°C)	Store in sterile 10mL aliquots at -20°C. Use once, then discard.

Normal human pooled plasma in 4% trisodium citrate	Sigma-Aldrich	P9523-5ML	2-8°C, sterile	Prepare BMC Control
recombinant human MPO	R&D Systems, Inc.	3174-MP-250	-80°C (6 months lyophilized; 3 months in solution)	Prepare BMC Control
Human MPO microplate	R&D Systems, Inc.	893941	up to 1 month at 2-8°C	96 wells; 12 strips of 8 wells
Human MPO conjugate		893942	up to 1 month at 2-8°C	
Human MPO Standard		893943	up to 1 month at 2-8°C	
Assay Diluent RD1-27		895245	up to 1 month at 2-8°C	
Calibrator Diluent RD6-58		895951	up to 1 month at 2-8°C	Diluent for serum, plasma or urine samples
Wash Buffer Concentrate		895003	up to 1 month at 2-8°C	
Color Reagent A		895000	up to 1 month at 2-8°C	
Color Reagent B		895001	up to 1 month at 2-8°C	
Stop Solution		895032	up to 1 month at 2-8°C	6mL of 2N sulfuric acid
plate sealers				4 adhesive strips

6.3. Reagent Comments

6.3.1. Seal unused wells with adhesive tape and return to foil pouch containing dessicant pack. Reseal. Store up to 1 month at 2-8°C.

6.3.2. MPO is detectable in saliva. Take care to prevent contamination of kit reagents while running this assay.

6.3.3. The human MPO standard provided in the kit was derived from human blood. Handle with universal precautions for biohazardous reagents.

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	
0.5-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

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 labeled 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 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 standards. A single patient's **batched** samples should be contained on one 96-well plate, not split over two plates, 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. 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 MPO Control

7.4.1.1. Solubilize lyophilized recombinant MPO to 1mg/mL (stock solution) in deionized water. Swirl to mix and let sit for 30 minutes at room temperature with occasional gentle mixing.

7.4.1.2. Dilute rMPO by adding 10 µL of stock solution to 990 µL HBSS in a 2.0 mL screw cap tube. This working solution is 10 µg/mL MPO.

7.4.1.3. Add 4ml of pooled human plasma to 36 mL of HBSS in a 50 mL conical tube. Mix gently, but thoroughly, with gentle swirling. Add 5 µL of MPO working solution for a final added concentration of 1.25 ng/mL MPO for the BMC Control. Discard remaining MPO working solution.

7.4.1.4. Make 200 µL aliquots of BMC control in 0.5 ml screw cap tubes. Label and store at -80°C. Controls are not diluted further in the assay. Controls are used once and excess is discarded

7.4.2. Store remaining rMPO stock solution (1mg/mL) in 10µL aliquots (about 24) at -80°C. It is stable for about 3 months.

7.4.3. Store remaining human pooled plasma in 100 µL aliquots at -80°C.

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 MPO 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 MPO 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. Reconstitute the lyophilized human MPO standard with deionized or distilled water. Refer to the kit vial label for reconstitution volume. This makes a stock solution of100 ng/mL MPO. Mix by gentle swirling to ensure complete reconstitution. Let sit for at least 15 minutes at room temperature with occasional gentle agitation prior to making dilutions. Vigorous agitation and foaming should be avoided.

Table V. Preparation of Standards

Standard #	Concentration (ng/mL)	Volume RD6-58 Diluent (µL)	Volume Standard (µL)	Final concentration in assay (ng/mL)
1	10	900	100 µL stock	3.33
2	5	500	500 µL #1	1.67
3	2.5	500	500 µL #2	0.83
4	1.25	500	500 µL #3	0.42
5	0.625	500	500 µL #4	0.21
6	0.313	500	500 µL #5	0.10
7	0.156	500	500 µL #6	0.05
8	0	500	0	0
(Volume, µL)		(4,400)		

7.7. Preparation of Unknowns (plasma samples)

7.7.1. Dilute plasma sample(s) 10-fold with calibrator diluent

7.7.1.1. For each unknown sample, add 180 µL Diluent RD6-58 to a polystyrene tube. Add 20 µL plasma. Vortex briefly to mix.

7.8. **ASSAY PROCEDURE**

7.8.1. Prepare all reagents, working standards, control and samples as directed in sections 7.4, section 7.5, section 7.6, and section 7.7.

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 100 µL of Assay Diluent RD1-27 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.

7.8.5. Incubate for 2 hours at room temperature on the shaker (0.12" orbit. 500±50rpm). 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 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 MPO Conjugate to each well using a multichannel pipettor. Cover with a new adhesive strip.

7.8.8. Incubate for 2 hours at room temperature on shaker (0.12" orbit. 500±50rpm). 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 7.8.6.

7.8.10. Prepare substrate as directed in section 7.5.3.1 within 15 minutes of use. Record mixing time. (Appendix 2, Section 3C). Add 200 µL of Substrate Solution to each well using a multichannel pipettor.

7.8.11. Incubate for 30 minutes at room temperature on benchtop and protect from light. 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): MPO ELISA MM/DD/YEAR PLATEX format (e.g., MPO 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).

8.0 DATA ANALYSIS

8.1. PRINCIPLE:

8.1.1. Signal data is converted to antigen (MPO) 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 an “MPO ELISA Analysis” program which tells the calculation-program the location of samples, standards, controls, the initial dilution and any serial dilutions. Wells designated as 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 analyte concentration for each sample is found by calculating the mean of the sample triplicate determinations based on the standard curve.

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: Control sample values must be within the established range for intra-assay variability (CV<15%; plates run on the same day) and inter-assay variability comparing plates run on different days (CV<30%).

8.2.6. If a sample has readings greater than the highest standard 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 analyte 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 lowest standard concentration and a sample has undetectable analyte 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 analyte concentration, do not report the result for the sample and re-analyze 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 signal of Standards and each sample well groupings.

8.3.2. For each analyte concentration, obtain the ‘signal’ by subtracting the average signal of the background wells from the average signal value of the corresponding wells that contain standards or unknowns.

8.3.3. Plot the background corrected signal values on the Y-axis and the logarithm of 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 analyte concentration.

9.0 REFERENCES

9.1. R&D User’s Guide for Human MPO 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, JK	3/7/2017
1.2	Draft	DSK, MT	5/24/2017
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			BMC CTL	BMC CTL	BMC CTL
G				S7T1			S15T4					
H				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			BMC CTL	BMC CTL	BMC CTL
G				S7C1			S15C3					
H				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

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	Exp Date
Normal human pooled plasma in 4% trisodium citrate	/ /		/ /
recombinant Human MPO	/ /		/ /
Human MPO microplate	/ /		/ /
Human MPO conjugate	/ /		/ /
Human MPO Standard	/ /		/ /
Assay Diluent RD1-27	/ /		/ /
Calibrator Diluent RD6-58	/ /		/ /
Wash Buffer Concentrate	/ /		/ /
Color Reagent A	/ /		/ /
Color Reagent B	/ /		/ /
Stop Solution	/ /		/ /

2. **Unknown Samples.** The first line gives an example with sample/patient ID, Module with Pre-analytic variable (PAV) and plasma dilution

Sample No	Sample/Patient ID	Module/PAV	Dilution (X)		
S Ex	TCP_0001	I / T2	10		
S1					
S2					
S3					
S4					
S5					
S6					
S7					
S8					
S9					
S10					
S11					
S12					
S13					
S14					
S15					
S16					
S17					
S18					
S19					
S20					

3. Plate Incubation: If not applicable, cross out.

A. Add clinical samples, controls, and standards, to the 96-well plate, cover plate, and incubate at room temperature for assay time. Record below.

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

B. Add Conjugate to the 96-well plate, cover plate, and incubate at room temperature for assay time. Record below.

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

C. Mix Substrate solution. Solution should be added within 15 minutes. Record below.

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:

4.1. SoftMax Pro Version:_____

4.2. 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
	Refrigerator (2-8^{o}C)			
	Freezer (-80^{o}C)			

6. Plate Map QC

a. Name of saved Excel data analysis workbook
_____

b. Plate Map Set Up QC

( ) Recommended Plate Map used. Circle one: A1 A2 A3 A4

( ) 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 (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 MPO ELISA.

BARC PRO 019: Thrombosis in Cancer Patients: Immunoassay of MPO in blood sample	• Perform ELISA with clinical samples, standards, and Controls • Using Versa Max Microplate reader, determine relative signal of all samples