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

BARC PRO 017 Plasma DNA SOP

Copy of version 2.1 (approved and current)

Last Approval or Periodic Review Completed	8/20/2018
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Location	TCP SOP SharePoint
Organization	Boston Medical Center

Description
Not final format

Comments for version 2.0 (last major revision)
Changed the way standards were made, in that the final standard is 125ng/ml instead of 25ng/ml to give a stonger curve with a greater signal to noise ratio.

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	3/31/2017	1.1	Chris Andry	Recorded when document uploaded to MediaLab
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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
1.1	Retired	Minor revision	3/30/2017	3/31/2017	7/19/2017
1.0	Retired	First version in Document Control	3/23/2017	3/23/2017	3/31/2017

BOSTON MEDICAL CENTER	Thrombosis in Cancer Patients Plasma DNA Assay		
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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 deoxyribonucleic acid (DNA) 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: When cells are injured or dying, they will release deoxyribonucleic acid (DNA) from their nucleus into the vascular spaces. This DNA can be quantified as a biomarker of cell injury using fluorescent dyes that bind to the DNA or intercalate between the bases (1,2). The assay approach here detects circulating double-stranded DNA (dsDNA) using a fluorescent DNA probe that does not penetrate cell membranes and whose fluorescence increases significantly upon binding to nucleic acids. The levels of plasma dsDNA are quantified by reference to a standard curve made with commercially available purified dsDNA.

2.2. CLINICAL SIGNIFICANCE: Increased plasma dsDNA concentrations are found in patients with acute coagulopathies including autoimmune thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, malignant disease and other conditions, and levels decrease during remission. The assay is based on Fuchs et al, *Blood* (120):1157-1164,2012.

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. It should be noted that phosphate salts and plasma proteins interfere with quantitation of dsDNA (2). Phosphate-free buffer and diluted plasma samples are used to minimize these influences.

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 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.

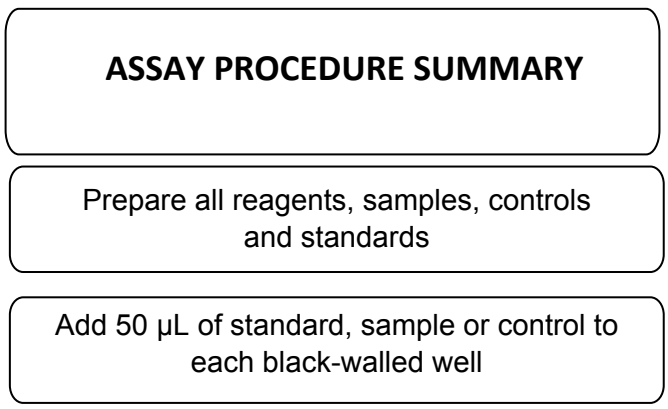
4.0 DEFINITIONS and ACRONYMS

4.1. Acronyms- see Table I.

Table I. Acronyms

Acronym	Name
dsDNA	double stranded DNA
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



Dilute Sytox Green fluorescent probe.
Add 50µL to each well & cover

Incubate 15 minutes, room temperature, covered.

Read Fluorescence. Ex485nm/Em525nm

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

Table II. Critical Reagents

Reagent	Vendor	Catalog #	Storage	Notes
Sytox Green	Invitrogen	S7020	-20>	250 µL of 5 mM stock solution in DMSO; protect from light.
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.
Salmon sperm DNA	Invitrogen	AM9680	-80+ 5 °C	stock 10mg/mL
Normal human pooled plasma in 4% trisodium citrate	Sigma-Aldrich	P9523-5ML	4-8°C, sterile	Prepare High and Low Controls

6.3. Reagent Comments

6.3.1. Sytox Green. Protect from light. The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation. Before refreezing, seal the vial tightly. Approximate fluorescence excitation/emission *maxima*: 504/523 nm when bound to DNA. Wavelengths in assay are optimized to Ex 485nm / Em 525nm. Avoid contact with detergents and high concentrations of divalent/monovalent cations.

6.3.2. Salmon sperm DNA. This sheared DNA has been treated with proteinase K to remove any contaminating nucleases, followed by organic extraction with phenol:chloroform and ethanol precipitation. Salmon sperm DNA is rigorously tested for RNAse and DNase contamination and is suspended in nuclease-free water in a 1mL tube at a concentration of 10mg/mL.

6.4. Consumables- See Table III

Table III. Consumables

Item	Range / Capacity	Quantity	Suggested Vendor / Catalog #
Pipet tips	200-1000 μL	1 box	USA Scientific/ 1126-7810
Pipet tips	50-200 μL	1 box	USA Scientific / 1120-8810
Pipet tips	2-20 μL	1 box	USA Scientific/ 1121-3810
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 tubes, sterile	15 mL		VWR 21008-918
Polypropylene tubes, sterile	50 mL		VWR 21008-951
96-well black-walled, clear bottom microplates [one plate for standards, controls, unknowns]			Nunc (#M33089)
aluminum foil			
Disposable reagent tray		at least 1	Sigma CLS4870

6.5. Equipment – see Table IV

Table IV. Equipment

Equipment	Range/Capacity	Manufacturer	Model	Serial No
Fluorometer	GEMINI XPS Dual-scanning microplate spectrofluorometer with thermal regulation and			

	SoftMax Pro acquisition and analysis software (Molecular Devices. LLC)			
Pipettor	200-1000 µL			
Pipettor	50-200 µL			
Pipettor	2-20 µL			
Pipettor	0.5-10 µL			
Multichannel Pipettor	50-300 µL			

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 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 High Control and Low Control for aliquot storage.

7.4.1.1. Thaw stock salmon sperm DNA solution (10mg/mL).

7.4.1.2. Pipette 18.0 mL HBSS into each of two (2) 50 mL conical tubes. Label one tube “High dsDNA Control”. Label the other “Low dsDNA Control”.

7.4.1.3. Pipette 2.0 ml Normal pooled human plasma (citrated) to each tube. Swirl briefly to mix. Document lot # of pooled plasma.

7.4.1.4. To High dsDNA Control tube: pipette 32 µL stock salmon sperm DNA. Swirl briefly to mix. Concentration is 16,000 ng/mL dsDNA. FINAL concentration in well is 8,000 ng/mL dsDNA.

7.4.1.5. To Low dsDNA Control tube: pipette 1.6 µL stock salmon sperm DNA. Swirl briefly to mix. Concentration is 800 ng/ml dsDNA. FINAL concentration in well is 400 ng/ml dsDNA.

7.4.1.6. Make 200 µL aliquots in screw cap tubes with O-ring. Each control volume will make about 100 aliquots. Label and put in -80^{o}C to freeze rapidly.

7.4.1.7. For remainder of normal human pooled plasma, make 100 µL aliquots (about 8 or 9) in screw cap tubes with O-ring. Label and put in -80^{o}C to freeze rapidly. These are useful if other dilutions are needed for controls.

7.4.1.8. Store frozen at -80^{o}C. Controls are used once and excess is discarded.

7.4.1.9. Actual measured control dsDNA concentrations will be increased by baseline dsDNA levels in normal human pooled plasma.

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

7.5.1.1. Thaw one aliquot HBSS (10ml)

7.5.1.2. Quickly thaw salmon sperm DNA stock solution (10mg/mL) at 37°C for 2 to 3 minutes.

7.5.1.3. For each plate, thaw one aliquot each of High dsDNA Control and Low dsDNA Control quickly (2-3 minutes) at 37°C, then keep at room temperature.

7.5.1.4. Prepare more as appropriate if multiple plates are run.

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

7.6.1. Prepare dsDNA Working Solution (20,000 ng/mL)

7.6.1.1. Pipette 1.245 mL HBSS into a 12x75mm polystyrene test tube. Add 5 µL of salmon sperm DNA stock solution (10mg/mL; source of dsDNA).

7.6.1.2. Vortex briefly. This is Standard #1 (40,000 ng/mL).

7.6.1.3. Prepare all other Standards according to **Table V** in labeled polystyrene test tubes. Add HBSS first to each tube. Then add volume of DNA working solution. Standards 2-5 are serial dilutions. Vortex each briefly and change pipette tips between standards.

Table V. Preparation of Standards

Standard #	Concentration (ng/mL)	Volume HBSS (µL)	Volume DNA (µL)	Final concentration in assay (ng/mL)
1	40,000	---	---	20,000
2	20,000	500	500 µL std #1	10,000
3	10,000	500	500 µL std#2	5,000
4	5,000	500	500 µL std#3	2,500
5	2,500	500	500 µL std#4	1,250
6	500	400	100 µL std#5	250
7	250	125	125 µL std#6	125
8	0	500	0	0
	(Volume, µL)	(4,145)		

7.7. Preparation of Unknowns (plasma samples)

7.7.1. Dilute plasma sample(s) 1:10 with HBSS

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

7.8. **Assay Procedure**

7.8.1. In each well of the 96 well black microplate, add 50µL of standard control or diluted plasma sample. Each is run in triplicate wells. Refer to Plate Map Design.

7.8.2. Prepare SytoxGreen Working Solution (working solution: 4µM) just before use.

7.8.2.1. Add 5.0 mL HBSS to a to a 15 mL conical tube. Add 4µL stock SytoxGreen (stock: 5mM in DMSO). Swirl to mix. If using a multipchannel pipettor, pour into a disposable reagent tray.

7.8.3. Add 50µl of SytoxGreen Working Solution to each well. The final concentration of fluorescent probe in each well is 2 µM. Plate wells should be pipetted in a consistently timed manner so that reactions experience relatively the same reaction incubation time.

7.8.4. Mix by tapping the microplate gently on the side

7.8.5. Transport plate to fluorometer and incubate with drawer closed. Protect from light.

7.8.6. Incubate at room temperature for 15 minutes. Record start and stop times (Appendix 2, Section 3c)

7.8.7. Insert microplate into spectrofluorometer. Read fluorescence using Excitation wavelength 485nm and Emission wavelength 525nm.

7.8.8. Save the resulting readings in dsDNA MM/DD/YEAR PLATE X format to a secure computer; recommended to label the file with the date and a unique assay identifier (Plate ID). 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. Fluorescence data is converted to analyte concentration with a computer program, SoftMax Pro. Acceptable results are obtained with computer programs using a standardized curve-fitting quadratic curve analysis.

8.1.2. The protocol calls for an analyte 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 fluorescence of the “blank wells” from the fluorescence 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 10 fluorescent units for all assay plates. If any blank wells are >10 fluorescent units, the assay should be examined for inappropriate results and should be re-assayed if no apparent causes are found.

8.2.2. Variability within 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. If more than 3 outliers are identified on one plate, the assay should be examined for inappropriate results and should be re-assayed if no apparent causes are found. This has to be documented in Appendix 2, section 7.

8.2.3. Standards: triplicate wells should have a CV of ≤15%, the lowest standard (125ng/mL) should have a signal near 5 fluorescent units (expected range 4-7) and the highest standard (20,000 ng/mL) should have a signal near 2,200 fluorescent units (expected range1,900-2,500) when analyzed with a quadratic function.

8.2.4. Sensitivity: sensitivity is defined as a signal:noise ratio with a range of 1.5-2.0. The ratio of the lowest standard value to the buffer blank value should be within this range when compared with the buffer (HBSS) blank values.

8.2.5. Quality Control: High and Low control sample concentrations must be within the established range for inter-assay variability comparing plates run on the same or 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 quadratic form 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. Fuchs, T.A., Kremer Hovinga, J.A., Schatzberg, D., Watner, D.D., and Lammie, B. 2012. Circulating DNA and myeloperoxidase indicate disease activity in patients with thrombotic microangiopathies. *Blood* 120:1157-1164.

9.2. Zhang, S.G., Yang, S., Vidyasagar, S., Zhang, M., Casey-Sawicki,K., Liu, C., Yin, L., Zhang, L., Cao, Y., tian, Y., et al. 2015. PicoGreen Assay of Circular DNA for Radiation Biodosimetry. *Radiation Research* 183:188-195..

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	Final approved draft	DSK, MT	3/27/2017
2.0	Modification of standards preparations	ERD	07/17/2017
2.1	Minor Corrections and Clarifications	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			CNTRL H	CNTRL H	CNTRL H
G				S7T1			S15T4			CNTRL L	CNTRL L	CNTRL L
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			CNTRL H	CNTRL H	CNTRL H
G				S7C1			S15C3			CNTRL L	CNTRL L	CNTRL L
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
Sytox Green	/ /		/ /
Hank's balanced salt solution (HBSS)	/ /		/ /
Salmon sperm DNA	/ /		/ /
Normal human pooled plasma in 4% trisodium citrate	/ /		/ /
	/ /		/ /
	/ /		/ /
	/ /		/ /
	/ /		/ /

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	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: If not applicable, cross out.

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

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

c. Add fluorescent probe, cover plate and incubate at room temperature for assay time. Record below.

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 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 cryovials

BARC PRO 017: Thrombosis in Cancer Patients: Immunoassay of plasma DNA in blood sample	• Perform assay with clinical samples, standards and controls • Determine relative signal of all samples, calculate concentrations