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

BARC PRO 018 BARC PRO 018 Nucleosomes ELISA

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Typos and clarifications

Approval and Periodic Review Signatures

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Approval	Primary Investigator	8/23/2017	1.3	Chris Andry, PhD Chris Andry	
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Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.4	Approved and Current	Minor revision	8/20/2018	8/20/2018	Indefinite
1.3	Retired	Minor revision	8/23/2017	8/28/2017	8/20/2018
1.2	Retired	Minor revision	8/23/2017	8/23/2017	8/28/2017
1.1	Retired	Minor revision	8/23/2017	8/23/2017	8/23/2017
1.0	Retired	First version in Document Control	3/23/2017	3/23/2017	8/23/2017

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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 nucleosomes 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. Murine monoclonal antibodies specific for human DNA (conjugated to peroxidase) and human histone (conjugated to biotin) are combined and added to the streptavidin-coated microplate. Standards, samples and control are pipetted into the wells. Peroxidase and 2'2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) substrate provide 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: Intact DNA and histone proteins are organized into units called nucleosomes, which form chromatin. Nucleosomes are expelled from the cell during cell inflammation, injury or death and they can be pro-thrombotic by activating some coagulation factors. Circulating nucleosome levels have been reported to be elevated in several different cancers and may play a role in the development of thrombosis in cancer patients.

2.3. SPECIMEN REQUIREMENT: Human platelet-poor plasma (citrate, heparin or EDTA anticoagulant). A minimum of 80 microliters (80µl) plasma is needed for each sample. Samples are run undiluted in the assay.

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.

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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**
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AU	Absorbance Units
CV	coefficient of variation
ID	Identification/ Identifier
LLQ	lower limit of quantification
NUC	Nucleosomes
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 3 hours to complete

Prepare all reagents, samples and standards.

Add 20 µl of Sample, Standard, Control or Blank to each well.
Add 80µL of Immunoreagent to each well and
Incubate for 2 hours at room temperature.

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Aspirate and wash 3 times

Add 100uL of ABTS substrate solution to each well.
Incubate for 15 minutes at room temperature.

Add 100 µl of ABTS Stop Solution

Read immediately at 405 nm

5.0 ENVIRONMENTAL HEALTH & SAFETY

5.1. Universal Safety Precautions 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. Use Cell Death Detection ELISA^{PLUS} kit (Roche Diagnostics GmbH, Sandhofer Strasse 116, 68305 Mannheim, Germany), Catalog number 11 774 425 001. Store up to 1 month at 2-8°C.

Table II. Critical Reagents

Reagent	Vendor	Catalog #	Storage	Notes
Cell Death Detection ELISA^{PLUS} kit	Roche Diagnostics	11 774 425 001	Up to 1 month at 2-8°C	

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5ddfcjYX UbX W ffYbh 9ZZYWhjY gHUfhb[, #&$#&$% "65F7 DFC $% fJYfgjcb %(Ł65F7 DFC $% Bi WYcgca Yg9@+G5

Anti-Histone-Biotin (bottle 1)	1 vial	11 774 425 001	Up to 1 month at 2-8°C	Reconstitute with 450µL distilled water
Anti-DNA-POD (bottle 2)	1 vial	11 774 425 001	Up to 1 month at 2-8°C	Reconstitute with 450µL distilled water
Positive Control (bottle 3)	1 vial	11 774 425 001	Up to 1 month at 2-8°C	Reconstitute with 450µL distilled water
Incubation Buffer (bottle 4)	100mL	11 774 425 001	Up to 1 month at 2-8°C	Ready-to-use
Substrate Buffer (bottle 6)	15mL	11 774 425 001	Up to 1 month at 2-8°C	Ready-to-use
ABTS Substrate Tablets (bottle 7)	need 3 tablets/plate	11 774 425 001	Up to 1 month at 2-8°C	Protect from light.
ABTS Stop solution (bottle 8)	100mL	11 774 425 001	Up to 1 month at 2-8°C	Ready-to-use
Microplate	12 strips with 8 wells each	11 774 425 001	Up to 1 month at 2-8°C	Reseal any unused strips with adhesive covers. Zip in plastic bag and store at 2-8°C
Normal human pooled plasma in 4% trisodium citrate	Sigma-Aldrich	P9523-5ML (order 3)	-80°C ± 5°C	Thaw rapidly at 37°C; Prepare BMC Controls

6.3. Reagent Comments

6.3.1. 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.
6.3.2. A lysis reagent (bottle 5) is included in the kit but not used for plasma samples
6.3.3. Sample diluent is a buffered protein base and preservatives.
6.3.4. Protect ABTS tablets from light.
6.3.5. Use double distilled water or distilled, deionized water for reagent reconstitutions
6.3.6. Incubation Buffer is also used for washes and the kit supply volume is limited. To accommodate, this procedure is modified from a usual ELISA to use the plate washer to only aspirate wells. Buffer is added manually with a multi-channel pipette for each wash. This buffer solution is subject to bubbles.
6.3.7. If turbidity or precipitate is visible in ABTS Stop Solution, warm to 37°C with occasional mixing until the solution is clear. Use at room temperature.

6.4. Consumables- See Table III

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5ddfcjYX UbX W ffYbH 9ZZYWhj Y gHUfhb[, #&$#&$%, "65F7 DFC $%, fJYfgjcb %(Ł65F7 DFC $%, Bi WYcgca Yg9@=G5

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	
2-mL tubes, O-ring screw cap, conical bottom, sterile	2 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
aluminum foil			

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
Orbital shaker	~300 rpm (gentle)			
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 and expiration date for all Critical Reagents in the Batch Record (Appendix 2, Section 1).

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5ddfcjYX UbX W ffYbh"9ZZYWj Y gUfhb[,#&$#&$% "65F7 DFC $% fJYfgcb %"(Ł65F7 DFC $% Bi WYcgca Yg9@6G5

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 expired.
6.6.3.2. Storage conditions and expiration dates for all Critical Reagents are provided on the package inserts.
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 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. Prepare BMC Control

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5ddfcjYX'UbX'W'ffYbh'9ZZYWh]j Y'gHUfh]b[', #&$#&$%, "'65F7 'DFC '$%, 'fJYfg]cb'%(Ł'65F7 'DFC '$%, 'Bi WYcgca Yg'9@=G5

7.4.1.1. Reconstitute each bottle of lyophilized human pooled plasma (Sigma Aldrich) with 5mL of distilled water. Three bottles will provide 15mL plasma. Allow to sit for 20 minutes at RT to solubilize with occasional gentle swirling to mix. Avoid excessive mixing or foaming. Alternatively, 15 mL of plasma can be provided from pooled healthy donors.

7.4.1.2. Pool the plasma in a 50 mL conical tube for a total volume of 15 mL. Add 300µL of Nucleosome Positive Control stock (provided in kit; bottle 3) to the pooled plasma. This should give an OD of ~0.6 at 405nm (~0.6 AU). Mix by swirling and make 100µL aliquots.

7.4.1.3. Label as BMC Nucleosome Control and put in -80°C to freeze rapidly. This should make about 150 aliquots.

7.4.1.4. 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. Prepare Immunoreagent

7.5.1.1. Re-suspend both anti-Histone antibody and anti-DNA POD antibody in 450µL of deionized water each. Allow to sit at RT for 15 minutes to ensure complete resuspension. Mix gently.

7.5.1.2. Pipette 445µl of anti-histone antibody into a 15mL conical tube. Add 445µL of anti-DNA POD antibody. To this equal mixture, add 8. 0 mL of incubation buffer. Label as with “Immunoreagent.” This volume is sufficient for one 96-well plate.

7.5.1.3. If only a few strips are being used, prepare sufficient volume (80µL/well) of Immunoreagent by mixing 1/20 volume Anti-DNA-POD with 1/20 volume anti-histone-biotin and 18/20 volumes Incubation Buffer.

7.5.1.4. Prepare Immunoreagent shortly before use. Do not store and discard any residual after use.

7.5.2. Prepare Wash Buffer

7.5.2.1. The kit’s Incubation Buffer is used as wash buffer. Calculate the required volume for wash buffer (250µL per well, three times) and pipette that volume of incubation buffer into a clean reagent reservoir. One full microplate will use ~75mL buffer. Label as “wash buffer.” Avoid agitation as solution is subject to bubbles.

7.5.3. **Prepare ABTS Substrate solution**

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7.5.3.1. Depending on the number of samples tested, dissolve 1,2 or 3 tablets in 5,10 or 15 mL of Substrate Buffer (bottle 6), respectively. A full 96-well plate will use 15 mL substrate. Prepare shortly before use. Protect from light.

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

7.6.1. Reconstitute Positive Control vial in 450µl of deionized water. Allow to solubilize at room temperature for at least 15 minutes and mix by occasional gentle inversion and swirling until all contents are completely dissolved. This is the Working Solution. Prepare a 1:10 dilution of the Working Solution for Standard #1 as shown in Table V. Standard #1 has a value of 10 Arbitrary Units (AU). Prepare 2-fold serial dilutions of Standard #1 using Incubation Buffer as shown in Table V. The standards are now ready for use in the assay.

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

Table V. Preparation of Standards

Standard #	Concentration AU	Volume Buffer (µL)	Volume Nucleosome (µL)	Final concentration in assay (AU)
1	10	360	40	2
2	5	200	200 of tube #1	1
3	2.5	200	200 of tube #2	0.5
4	1.25	200	200 of tube #3	0.25
5	0.625	200	200 of tube #4	0.125
6	0.313	200	200 of tube #5	0.625
7	0	200	0	0
	(Volume, µL)	(1560)		

7.7. Preparation of BMC Nucleosome Control

7.7.1. Thaw BMC Nucleosome Control rapidly at 37°C. Allow the control to sit on benchtop before use.

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5ddfcjYX UbX Wi ffYbh" 9ZZYWhjj Y gHUfhjb[, #&$#&$%, "65F7 DFC $%, fJYfgjcb %(Ł65F7 DFC $%, Bi WYcgca Yg 9@G5

7.8. Preparation of Unknowns (plasma samples)

7.8.1. Plasma samples will used undiluted in this assay

7.8.1.1. Thaw plasma samples rapidly at 37°C, then place all plasma samples in rack on benchtop until ready for use.

7.9. **Assay Procedure**

7.9.1. Prepare all reagents, working standards, controls and samples as directed in section 7.5-7.8

7.9.2. Remove excess microplate strips from the plate frame, cover with adhesive plate sealer, return them to the pouch containing the desiccant pack, and reseal.

7.9.3. To each well, add 20 µL Standard, Control, Unknown, or Incubation Buffer (blank wells) as shown in the Plate Map (Appendix 1).

7.9.4. Add 80 µL Immunoreagent to each well.

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

7.9.6. Incubate for 2 hours at room temperature on an orbital shaker with gentle shaking (~300rpm). Record the date, start time, and incubation temperature in the Batch Record (Appendix 2, Section 3a).

7.9.7. Wash

7.9.7.1. Following incubation, aspirate the plate using a plate washer (for the BioTek Plate Washer, use the *Nuc Aspirate*, option #3 under aspiration, program). Remove plate from the washer and blot quickly on paper towels. Immediately wash the plate manually 3 times.

7.9.7.1.1. Pipette 250 µL Incubation Buffer to each well using a multichannel pipettor, let sit for 30 seconds, then aspirate with the plate washer and blot. Repeat 2 more times for a total of 3 washes. Rotating the plate 180 degrees between wash steps may improve assay precision.

7.9.7.2. After the final 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.

7.9.8. Adding Substrate

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7.9.8.1. Gently dispense solubilized ABTS substrate into a disposable reagent reservoir.

7.9.8.2. Add 100 µL Substrate to each well using a multichannel pipettor. Cover the plate with a new plate sealer and aluminum foil to protect from light. Incubate at room temperature for 15 minutes on orbital shaker.

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

7.9.9. Adding Stop Solution

7.9.9.1. Add 100 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.

7.9.10. Determine Optical Density (OD)

7.9.10.1. Determine the optical density of each wells within 30 minutes, using a microplate reader set to 405 nm.

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

7.9.11. 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 analyte 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 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 will contain buffer (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.

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5ddfcjYX UbX W ffYbh 9ZZYWhj Y gHUfh]b[, #&$#&$%, "65F7 DFC $%, fJYfg]cb %(Ł65F7 DFC $%, Bi WYcgca Yg9@=G5

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 (CV<30%; comparing plates run on different days).

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

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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. Roche User Manual for Cell Death Detection ELISA PLUS Kit (version 15; March 2016).

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	MPT, DSK	06/14/2017
1.3	Draft ; minor clarifications	MPT, DSK	08/02/2017

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1.4	Minor Clarifications, typos, formatting	BET,DSK,ERD,MPT	8/1/2018

7cbhfc YX Wcdm8 %(-$--" DU[Y % cZ% ! DU[Y 7ci bh

5ddfcjYX UbX W ffYbh"9ZZYWhjj Y gHUfhb[, #&$#&$% "65F7 DFC $% fJ Yfgjcb %"(Ł65F7 DFC $% Bi WYcgca Yg9@-G5

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 Diluent or Buffer 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	Blank	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	Blank	Blank

7cbhfc``YX`Wcdm=8`%(-$--"DU[Y`%(`cZ%`!DU[Y`7ci bh

5ddfcjYX UbX fYYbh 9ZZYWjY gUfh[, #&$#&$% "65F7 DFC $% fJYfg]cb %"(Ł65F7 DFC $% Bi WYcgca Yg9@=G5

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
Anti-Histone-Biotin	/ /		/ /
Anti-DNA-POD	/ /		/ /
Positive Control	/ /		/ /
Incubation Buffer	/ /		/ /
Lysis Buffer	/ /		/ /
Substrate Buffer	/ /		/ /
ABTS Substrate Tablet	/ /		/ /
ABTS Stop solution	/ /		/ /
Microplate	/ /		/ /
Normal human pooled plasma	/ /		/ /

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)**		

7cbhfc``YX`Wcdm`8`%(-$--"`DU[Y`%)`cZ%-`!`DU[Y`7cibh

5ddfcjYX UbX WffYbh 9ZZYWhjY gUfhb[, #&$#&$% "65F7 DFC $% fJYfg]cb %(Ł65F7 DFC $% Bi WYcgca Yg9@=G5

S Ex	TCP_0001	I / T2	5		
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)
/ /	:	:	

5ddfcjYX UbX W ffYbh 9ZYWhj Y gHUfhb[, #&$#&$%"65F7 DFC $% fJ Yfgjcb %(Ł65F7 DFC $% Bi WYcgca Yg9@G5

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)
/ /	:	:	

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°C)			
	Freezer (-80°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: A1.1 A1.2

( ) Alternative plate map used; cells copy and pasted individually to the Plate Layout QC worksheet.

Reason: ____

7cbhfc``YX`Wcdm=8`%(-$--"`DU[Y`%+`cZ%-`!`DU[Y`7ci bh

5ddfcjYX UbX WiffYbh"9ZZYWhjY gHUfhjb[,#&$#&$%""65F7 DFC $%' fJYfgjcb'%(L65F7 DFC $%' Bi WYcgca Yg9@=G5

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: ____________________

7cbhfc`YX`Wcdm8 %(-$--"DU[Y %cZ% !DU[Y 7ci bh

5ddfcjYX UbX W ffYbH 9ZYWhj Y gHUfhb[, #&$#&$% "65F7 DFC $% fJYfgjcb %"(Ł65F7 DFC $% Bi WYcgca Yg9@=G5

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 Nucleosome ELISA.

BARC PRO 018: Thrombosis in Cancer Patients: Immunoassay of Nucleosomes in blood sample	• Perform ELISA with clinical samples, standards and BMC control • Using Versa Max Microplate reader, determine relative signal of all samples

7cbhfc YX Wcdm8 %(-$--"DU[Y % cZ% !DU[Y 7ci bh