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

BARC PRO 025 Souble Thrombomodulin SOP

Copy of version 1.3 (approved and current)

Last Approval or Periodic Review Completed 2/13/2018

Next Periodic Review Needed On or Before 2/13/2019

Effective Date 8/20/2018

Controlled Copy D 127447

Location CP SharePoint

Organization Boston Medica Center

Comments for version 1.2 (last major revision)
nitia version

Comments for version 1.3 (this revision)
ypos and c arifications

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approva	QA Review	8/20/2018	1.3	ERDuffy E izabeth Duffy	

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.3	Approved and Current	Minor revision	8/20/2018	8/20/2018	ndefinite
1.2	Retired	nitia version	2/12/2018	2/13/2018	8/20/2018

1.0 PURPOSE AND SCOPE

1.1. The purpose of this SOP is to provide standardized instructions and guidance for measurement of Soluble Thrombomodulin (sTM) in human plasma in the Pathology and Laboratory Medicine Department of Boston Medical Center (BMC).

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

2.0 OVERVIEW

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

2.2. CLINICAL SIGNIFICANCE:
Thrombomodulin (TM) is a multi-functional receptor expressed primarily by endothelial cells. Cellular TM expression levels inversely correlate with migration properties of tumor cells and loss of TM expression and associated function is associated with tumor growth and higher metastatic potential in colorectal, lung, prostate, melanoma and breast cancers. Thrombomodulin's luminal amino-terminal domains are released into the circulation as a soluble biomarker (sTM) upon inflammatory stimulus or injury of the parent cells.

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

3.0 RESPONSIBILITY

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

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

3.3. It is the responsibility of the project staff designated by the PI or 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
Soluble Thrombomodulin	sTM
CV	coefficient of variation
HBSS	Hank's balanced salt solution
ID	Identification/ Identifier
LLQ	lower limit of quantification
PBS	phosphate buffered saline
SD	standard deviation
SOP	standard operating procedure
UA	unanalyzable
ULQ	upper limit of quantification

4.2 Assay Procedure Summary

Prepare all reagents, samples and standards.

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

Add 50 µl of **1X Biotinylated Detection Antibody** to all wells.

Cover and incubate at room temperature for 1 hour.

Wash the plate with 300µL 1x **wash buffer** three times.

Add 100µL of **1x** Streptavidin-**HRP-Conjugated Antibody** to all wells.

Cover and incubate at room temperature for 30 minutes.

Repeat previous Wash step.

Add 100µL of **TMB Substrate Solution** to each well.

Incubate at room temperature for 15 minutes
Protect from light.

Add 100µL of **Stop Solution** to each well. Read absorbance at 450nm with wavelength correction at 620nm

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 023 (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

6.2.1. Human sTM ELISA kit (Catalog number: CDK004A, Cell Sciences, Inc, Canton, MA 02021, USA). Store all kit components at 2-8^{o}C. The substrate should never be

frozen. Once individual reagents are opened, it is recommended that the following kit components are not stored: Standards, Controls, Biotinylated Secondary Antibody, Streptavidin-HRP. The following kit components can be stored for up to one week at 2-8°C after opening: Wash Buffer and Standard Diluent Buffer. Unused strip plate wells should be stored at 2-8°C in a sealed bag containing desiccant in order to minimize exposure to moisture. Do not use the kit beyond its expiration date. See Table II for kit components.

6.2.2. Other critical reagents see Table II.

Table II. Critical Reagents

Reagent	Vendor	Catalog #	Storage	Notes
Hank's balanced salt solution (HBSS)	Thermo Fisher Scientific	14025-092	Keep stock solution bottles at room temp (~25°C)	Store in sterile 10mL aliquots at -80 ± 5 °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 Thrombomodulin	R&D Systems, Inc.	3947-PA-010	6 months from date of receipt, -80°C as supplied	10 µg, supplied in Tris saline solution. Prepare BMC Control
Pre-coated 96-well strip plate	Cell Sciences, Inc. Canton, MA	CDK004A	2-8°C, supplied in ELISA kit	1 plate
Standard: 20 ng/ml			2-8°C, supplied in ELISA kit	2 Vials, Reconstitute in 880 µl Standard Diluent (per vial)*
Control			2-8°C, supplied in ELISA kit	2 Vials, Reconstitute in 1.5 ml Standard Diluent (per vial)*
Standard Diluent (Buffer)			2-8°C, supplied in ELISA kit	1 vial (25 ml) 10x Concentrate, dilute in distilled water
Biotinylated anti-Human sTM			2-8°C, supplied in	1 vial (0.4 ml) Dilute in

antibody			ELISA kit	Biotinylated Antibody Diluent
Biotinylated Antibody Diluent			2-8°C, supplied in ELISA kit	1 vial (7 ml), Ready to use
Streptavidin-HRP			2-8°C, supplied in ELISA kit	2 vials (5 µl/vial), Add 0.5 ml of Streptavidin-HRP Diluent/vial prior to use
Streptavidin-HRP Diluent			2-8°C, supplied in ELISA kit	1 vial (23 ml), Ready to Use.
Wash Buffer			2-8°C, supplied in ELISA kit	1 vials (10 ml), 200x Concentrate, dilute in distilled water
TMB Substrate			2-8°C, supplied in ELISA kit	1 vial (11 ml), Ready to Use
H_2SO_4 stop reagent			2-8°C, supplied in ELISA kit	1 vial (11 ml), Ready to Use
Plastic Plate Sealers			supplied in ELISA kit	2 Sealers

* Check instruction sheet for any changes in reconstitution volumes

6.3. Reagent Comments

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 35	
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
Sealing tape for 96 well plates			Thermo Fisher 15036
Disposable reagent reservoirs			Thermo Fisher 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
Refrigerator	2-8°C			
-80°C Freezer	-80 ± 5 °C			

6.6. Reagent storage and stability

6.6.1. Record the date of receipt, lot number, and 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. Prepare BMC Control for aliquot storage.

7.4.1.1. Recombinant sTM protein is supplied as 10µg in a filtered Tris-saline buffer (~20 µL). This stock sTM concentration will vary, but will be near 500µg/mL. Record the concentration on the vial and dilute to a final concentration of 1ug/mL in 500µL final volume using HBSS. For example, if the stock is 500µg/ml, add 1µL stock sTM to 499µL HBSS. Label this vial "sTM working solution." The concentration of this working solution is 1 ug/mL.

7.4.1.2. Reconstitute lyophilized human plasma with 5mL DI water. Allow to sit for at least 15 minutes with gentle mixing. Do not shake.

7.4.1.3. Transfer 4.8 mL reconstituted plasma into a 15 mL conical tube labeled "sTM Control."

7.4.1.4. Add 4.752 mL HBSS to sTM control tube and mix gently by swirling.

7.4.1.5. Transfer 48µL of sTM working solution to the sTM control tube containing diluted plasma. Cap the tube and mix by gentle inversion. The final concentration of sTM will be 5ng/mL increase over initial levels present in the diluted plasma.

7.4.1.6. Aliquot sTM control solution in 400µL aliquots into 0.5mL cryovials labeled "BMC sTM Control." This will make about 24 aliquots.

7.4.1.7. For remainder of normal human pooled plasma, make 100 µL aliquots (about 1 or 2) in screw cap tubes with O-ring. Label and put in -80°C freezer to freeze rapidly.

7.4.1.8. Store frozen at -80°C. BMC 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 Wash Buffer**

7.5.1.1. Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2L graduated cylinder. Bring final volume to 2L with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2-8˚C for up to 1 week.

7.5.2. **Preparation of Standard Diluent Buffer**

7.5.2.1. Add the contents of the vial (a 10x concentrate) to 225 ml of distilled water in a clean container before use and mix. This solution can be stored at 2-8˚C for up to 1 week.

7.5.3. **Preparation of ELISA Kit Control**

7.5.3.1. A control sample is provided with the kit. Re-constitute with 1.5ml of Standard Diluent (double check the kit instruction sheet for reconstitution volume). Let sit for at least 15 minutes at room temperature to solubilize. Mix by gentle swirling. Avoid vigorous mixing or foaming. The final concentration will be stated on the vial. Record concentration in the Batch Record (Appendix 2, Section 1).

7.5.4. **Preparation of Biotinylated Antibody (immediately before use)**

7.5.4.1. Add 6.360 mL biotinylated antibody diluent to a 15 mL conical tube. Label "b-Antibody". Just before use, add 240µL biotinylated anti-sTM antibody. Cap and mix gently by inversion. Avoid vigorous mixing or foaming. Add to wells immediately.

7.5.5. **Preparation of Streptavidin-HRP (immediately before use)**

7.5.5.1. Centrifuge stock tube briefly in a microfuge before dilution to collect the volume (5µL). Dilute by adding 500µL Streptavidin-HRP diluent immediately before use.

7.5.5.2. Add 10 mL of Streptavidin-HRP diluent to a 15 mL conical tube. Label "Streptavidin-HRP conjugate"

7.5.5.3. Transfer 150 µL diluted Streptavidin-HRP to the Streptavidin-HRP conjugate tube. Cap and mix by gentle inversion. Add to wells immediately.

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

7.6.1. Standard vial(s) must be reconstituted in 880 µl Standard Diluent (per vial) (check the instruction sheet for reconstitution volume) immediately prior to use. Let stand for at least 15 minutes at room temperature to solubilize. Avoid vigorous mixing or foaming. This reconstitution gives a stock solution of 20 ng/ml sTM. Mix the reconstituted standard gently by inversion only. If both vials are needed, reconstitute both, then pool and mix for one Standard solution vial.

7.6.2. Label eight 12x75 mm test tubes, numbered 1 through 8, for the sTM standards. Using the reconstituted Standard, prepare standards #1-7 with two-fold serial dilutions for final concentrations of 20 to 0.313 ng/mL in sample diluent (Table V: Preparation of Standards). Tube 8 will contain only sample diluent.

7.6.3. Add 100 µL of each Standard to wells immediately.

Table V. Preparation of Standards

Standard #	Concentration (ng/mL)	Volume Standard Diluent (µL)	Volume Standard (µL)	Final concentration in assay (ng/mL)
1	20	0	800	13.3
2	10	400	400 tube #1	6.7
3	5	400	400 tube #2	3.3
4	2.5	400	400 tube #3	1.7
5	1.25	400	400 tube #4	0.8
6	0.625	400	400 tube #5	0.4
7	0.313	400	400 tube #6	0.2
8	0	400	0	0

7.7. **Preparation of Unknowns (plasma samples)**

7.7.1. Dilute plasma sample(s) with Standard Diluent

7.7.1.1. Thaw plasma samples at room temperature. **Do not thaw at 37ºC.**

7.7.1.2. For each unknown sample, add 200 µL Standard Diluent to a polystyrene tube. Add 200 µL plasma. Vortex briefly to mix.

7.8. **Assay Procedure**

7.8.1. In each well of the 96 well plate, add 100µL of standard, control, blank or diluted plasma sample. Each is run in triplicate wells. Refer to Plate Map Design. (Appendix 1)
7.8.2. Make dilution of biotinylated anti-sTM antibody and add 50 µl to all wells.
7.8.3. Cover with an adhesive sealer and incubate at room temperature for 1 hour.
7.8.4. Wash three times
7.8.4.1. Remove the cover and wash the plate as follows:
7.8.4.1.1. Aspirate the liquid from each well.
7.8.4.1.2. Dispense 0.3ml of 1x washing solution into each well.
7.8.4.1.3. Aspirate the contents of each well.
7.8.4.1.4. Repeat another two times
7.8.5. 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:	300 µL/well	300 µL/well

7.8.6. Make the dilutions of streptavidin-HRP and add 100µl of Streptavidin-HRP solution into all wells. Do not allow the wells to dry.
7.8.7. Cover with an adhesive seal and incubate at room temperature (18 to 25°C) for 30 minutes.
7.8.8. Wash three times
7.8.8.1. Repeat wash step 5.
7.8.9. Add 100 µL of ready-to-use TMB Substrate Solution into all wells. Cover plate with foil to avoid exposure to light.
7.8.10. Incubate 15 minutes at room temperature.
7.8.11. Add 100 µL of H_2SO_4 Stop Reagent into all wells.
7.8.12. Read the absorbance value of each well using 450 nm as the primary wavelength and optionally 620 nm as the wavelength correction if available (610nm to 650nm is acceptable). Absorbance should be read immediately after adding Stop Reagent.
7.8.13. Save the resulting readings in BIOMARKER NAME 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. 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 (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 (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 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. Cell Sciences User Manual for Human Soluble Thrombomodulin ELISA kit.

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	MPT	12/29/2017
1.1	Draft	DSK	01/09/2018
1.2	Draft	DSK, MPT	01/26/2018
1.3	Minor Revisions	BET,DSK,ERD,MPT	08/01/2018

APPENDIX 1: PLATE MAP DESIGN: Patient samples from Module I, <1 hour will be assayed with similar samples from different donors on this 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 Plate Design (Baseline Donor Sample): Room Temperature Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A		STDS		S1T1			S9T1			S17T1		
B				S2T1			S10T1			S18T1		
C				S3T1			S11T1			S19T1		
D				S4T1			S12T1			S20T1		
E				S5T1			S13T1			S21T1		
F				S6T1			S14T1			S22T1		
G				S7T1			S15T1			BMC CTL	BMC CTL	BMC CTL
H				S8T1			S16T1			Kit CTL	Kit CTL	Kit CTL

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
96 well microtiter strip plate	/ /		/ /
Standard: 20 ng/ml	/ /		/ /
Kit Control Concentration:	/ /		/ /
Standard Diluent	/ /		/ /
Biotinylated Antibody	/ /		/ /
Biotinylated Antibody Diluent	/ /		/ /
Streptavidin-HRP Conjugate	/ /		/ /
Streptavidin-HRP diluent	/ /		/ /
Wash Buffer	/ /		/ /
TMB substrate	/ /		/ /
Stop Solution			

Normal human pooled plasma in 4% trisodium citrate	/ /		/ /
Recombinant Human Thrombomodulin	/ /		/ /
Hank's Balanced Salt 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	20		
S1					
S2					
S3					
S4					
S5					
S6					
S7					
S8					
S9					
S10					
S11					
S12					
S13					
S14					
S15					
S16					
S17					
S18					
S19					
S20					

S21					
S22					

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 1 hour. Record below.

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

b. Add Streptavidin-HRP Conjugate to the 96-well plate, cover plate, and incubate at room temperature for 30 minutes. Record below.

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

c. Add Substrate, cover plate and incubate at room temperature for 15 minutes. 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^{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 sTM ELISA.

BARC PRO 025: Thrombosis in Cancer Patients: Immunoassay of soluble Thrombomodulin in blood sample	• Perform ELISA with clinical samples, sTM standards, sTM controls • Using Versa Max Microplate reader, determine relative signal of all samples