National Clinical Target Validation Laboratory (NCTVL)

Applied Developmental Directorate

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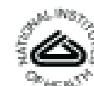

Change History

Revision	Approval Date	Description	Originator	Approval
--	10/16/2006	New Document	YZ	JJ
A	7/17/2007	Revision for Phase 1	YZ	JJ
B	10/14/2008	Merge Tissue Processing (SOP34506) with protein assay (SOP340510)	KG	JJ
C	12/01/2008	Revision of SOP based on first PAR Immunoassay Training Course to clarify sample processing steps, update SOP Web site, SOP title, and move reagent preparation to Batch Record for technician sign-off	YZ	JJ
D	8/10/2009	Update SOP references, separated BCA sample tables to Appendix 2, added PAR IA processing flow chart	YAE	JJ
E	4/8/2011	Incorporated BCA assay into SOP work flow, incorporated Appendix 3 into Batch Record and, updated Sections 5.0 and 6.0	YAE	JJ
F	1/8/2013	Stock lysate no longer diluted to 1 µg/µL for immunoassay, minimal stock lysate total protein requirement of 0.16 µg/µL defined. Sample Information and BCA Assay reporting tables combined. BCA assay section modified to only prepare two dilutions.	KFG, YAE	KFG

Please check for revision status of the SOP at

http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

and be sure to use the current version.

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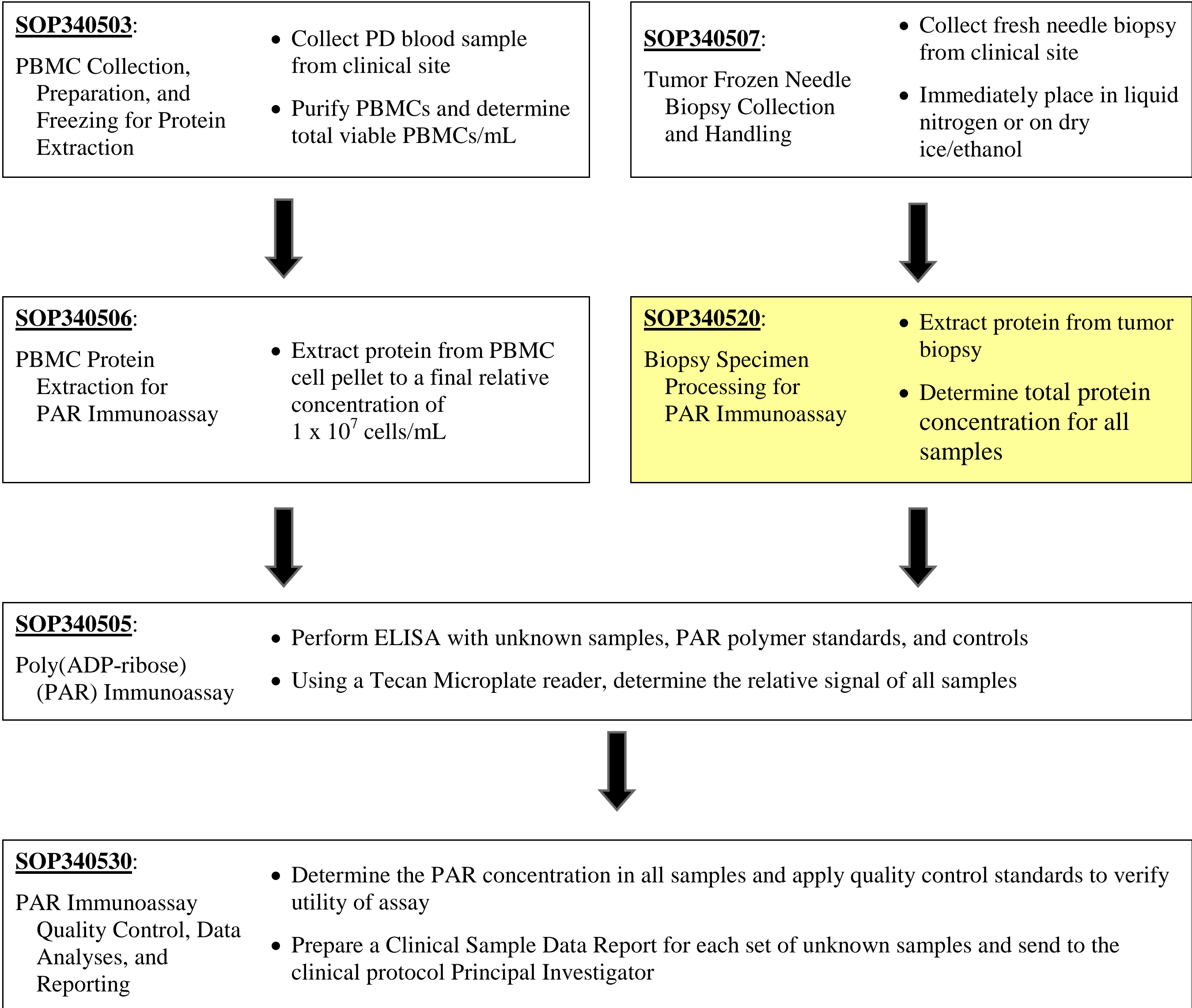
OVERVIEW OF PAR IMMUNOASSAY SPECIMEN PROCESSING



1.0 PURPOSE

Standardize the method for preparing lysates from biopsy samples to enable quantification of poly(ADP-ribose) (PAR) levels with an enzyme-linked immunosorbent assay (ELISA) in pharmacodynamic (PD) studies of PAR polymerase (PARP) inhibitors and/or chemotherapeutic agents.

2.0 SCOPE

This procedure applies to all personnel involved in measurement of PAR as a PD marker during clinical trials and in the preparation of samples for the analysis of PAR levels by the PAR Immunoassay (SOP340505). The goal of the SOP and associated training is to ensure consistency in PAR measurement across samples and clinical sites.

3.0 ABBREVIATIONS

BCA	=	Bicinchoninic Acid
BSA	=	Bovine Serum Albumin
CEB	=	Cell Extraction Buffer
DCTD	=	Division of Cancer Treatment and Diagnosis
ELISA	=	Enzyme-Linked ImmunoSorbent Assay
ID	=	Identifier
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
PADIS	=	Pharmacodynamic Assay Development and Implementation Section
PAR	=	Poly(ADP-ribose)
PARP	=	Poly(ADP-ribose) Polymerase
PBMC	=	Peripheral Blood Mononuclear Cells
PD	=	Pharmacodynamic
PI	=	Protease Inhibitor
PMSF	=	Phenylmethanesulfonyl Fluoride
QC	=	Quality Control
RT	=	Room Temperature
SDS	=	Sodium Dodecyl Sulfate
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The PAR Immunoassay (SOP340505) has been developed to measure the effect of PARP inhibitors and/or chemotherapeutic agents on PAR levels in a variety of biospecimen types, including peripheral blood mononuclear cells (PBMCs) and tissue/tumor biopsies. An ELISA is used to first capture PAR from total cell extracts on plates coated with a PAR capture monoclonal antibody. The captured protein is then detected using a PAR polyclonal detection antibody followed by addition of an HRP-conjugate to allow chemiluminescent readout and quantitation of PAR levels.

5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor	The Laboratory Director/Supervisor, directs laboratory operations, supervises technical personnel and reporting of findings, and is responsible for the proper performance of all laboratory procedures. The Laboratory Director/Supervisor also oversees the personnel running SOPs within the laboratory and is responsible for ensuring that only certified and experienced personnel handle clinical samples.
Certified Assay Operator	A Certified Assay Operator may be a Laboratory Technician/ Technologist, Research Associate, or Laboratory Scientist who has been certified through training on this SOP. The Certified Assay Operator works under the guidance of the Laboratory Director/Supervisor. This person performs laboratory procedures and examinations in accordance with the current SOP(s), as well as any other procedures conducted by a laboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

*Depending on the laboratory, one person may have multiple roles.

5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented training and qualification on this SOP prior to the actual handling and processing of samples from clinical trial patients. The Laboratory Director/Supervisor is responsible for ensuring the Certified Assay Operator running the SOP has sufficient experience to handle and analyze clinical samples.

5.2 The Certified Assay Operator responsible for conducting the assay is to follow this SOP and complete the required tasks and associated documentation. The Batch Record (Appendix 1) must be completed in ***real-time*** for each experimental run, with each page ***dated and initialed*****,** and placed with the clinical sample information.

5.3 Digital versions of the sample table in the Batch Record (Appendix 1, Section 2) can be created for logging sample information as long as all column information exactly matches the table in the Batch Record. A copy of the completed, digital sample tables must be printed and attached to the Batch Record in order to maintain a complete audit trail.

5.4 The responsible personnel are to check the DCTD Biomarkers Web site (http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm) to verify that the most recent version of the SOP for the assay is being used.

6.0 MATERIALS AND EQUIPMENT REQUIRED

6.1 Pipettors (200-1000 µL, 50-200 µL, 2-20 µL) and tips
6.2 Multichannel pipettor (50-200 µL) and reservoirs
6.3 Reagent reservoirs (e.g., Fisher Scientific, Cat#: 21-381-27C)
6.4 1.5-mL Sarstedt o-ring screw cap tubes (e.g., Sarstedt, Cat#: 72.692.005)
6.5 2-mL Sarstedt o-ring screw cap, skirted tubes (e.g., Sarstedt, Cat#: 72.694.006)
6.6 50-mL polypropylene tubes (e.g., Becton Dickinson, Cat#: 352098)
6.7 High-quality fine-tipped mincing scissors
6.8 0.4-mL 96-well Flat Bottom Bacti Plate (Fisher Scientific, Cat#: 12-565-361)
6.9 Acetate plate sealers (Thermo Scientific, Cat#: 3501)
6.10 Cryogenic marker (e.g., Thermo Scientific, Cat#: 4000221)
6.11 81-place freezer storage boxes (e.g., Fisher Scientific, Cat#: 12-565-182)
6.12 Ice bucket

6.13 UltraPure DNase/RNase-free distilled water (e.g., Invitrogen, Cat#: 10977-015) or Milli-Q water
6.14 Protease Inhibitor Cocktail (Sigma-Aldrich, Cat#: P-2714 or Roche, Cat#: 11697498001)
6.15 Phenylmethanesulfonyl fluoride solution, 0.1 M (PMSF; Sigma-Aldrich, Cat#: 93482-50ML-F)
6.16 Cell Extraction Buffer (CEB; Invitrogen, Cat#: FNN0011)
6.17 20% sodium dodecyl sulfate (SDS; e.g., Sigma-Aldrich, Cat#: 05030-500ML-F)
6.18 BCA Protein Assay Kit (Thermo Scientific Pierce, Cat#: 23227 or 23225)
6.19 Liquid nitrogen or dry ice/ethanol bath

6.20 Sorvall Fresco microcentrifuge (Fisher Scientific)
6.21 Vortex Genie 2 (Daigger, Cat#: EF3030A)
6.22 Ultrasonic Processor (Cole-Parmer Instruments, Model#: CP 130PB-1)
6.23 Infinite® 200 or Infinite 200Pro Microplate Reader (Tecan US)
6.24 100°C heat block or boiling water bath
6.25 37°C incubator (e.g., Fisher Scientific, Cat#: 11-690-516D)
6.26 -20°C and -80°C freezer
6.27 2°C to 8°C refrigerator

6.28 Microsoft Excel (2003 or newer), or equivalent
6.29 Biopsy specimens processed following SOP340507 (Tumor Frozen Needle Biopsy Specimen Collection and Handling)

*If instruments and/or reagents differ from those specified above, the Certified Assay Laboratory processing the clinical specimens must prove their comparability or equivalence to those recommended using the manufacturer's specifications and experimental validation data.

7.0 OPERATING PROCEDURES

7.1 All reagents for an individual assay are to be prepared for use in one experimental run, and only in the amounts required for the specific assay. All excess reagents are to be discarded following appropriate safety procedures. Process a single patient's samples, **batched**, to ensure consistent sample handling.

7.2 Record the name and certification number of the Certified Assay Operator and the facility running the SOP in the Batch Record (Appendix 1). Include reference clinical protocol number(s), if applicable.

7.3 Record equipment make, model, and serial numbers that will be used in the assay in the Batch Record (Appendix 1, Section 1A) and prepare the reagents outlined (Appendix 1, Section 1B). **Note**: Do not add protease inhibitors or PMSF (PIs) to Cell Extraction Buffer (CEB) until noted in the SOP and do not prepare the BCA Working Reagent until noted in the SOP.

7.4 Tissue Lysis

7.4.1 Fill in the Sample Information Table in the Batch Record (Appendix 1, Section 2) with the Sample/Patient ID for each biopsy.

7.4.1.1 The sample/patient ID should include the CTEP protocol number followed by a unique patient identifier and a sequential specimen ID (NCI tumor biopsies for PD sampling are series 500).

7.4.2 Prepare fresh CEB with protease inhibitors and PMSF (**with** PIs) as outlined in the Batch Record (recipe in Appendix 1, Section 1B). Keep on ice.

7.4.3 Place frozen needle biopsy samples on ice.

7.4.4 One sample at a time, so that biopsy is still frozen during mincing step:

7.4.4.1 Add 400 µL of ice-cold CEB (**with** PIs) and keep sample in ice. Record the volume CEB (**with** PIs) used for each sample in the Sample Information Table (Appendix 1, Section 2).

7.4.4.2 Immediately mince the still-frozen tissue with fine scissors.

7.4.4.3 Vortex the tube at maximum speed for 10 sec, mince the tissue again, and then vortex at maximum speed for an additional 10 sec.

7.4.4.4 Repeat with the next biopsy.

7.4.5 Incubate tubes with the minced tissue on ice for a minimum of 5 min; record the start and stop times in the Batch Record (Appendix 1, Section 3).

7.4.6 Sonicate the tissue at an output of 02-03 watts for 15 to 30 sec; repeat 3 times. Keep the tube on ice while sonicating and avoid foaming of specimens. Record the sonicator settings in the Batch Record (Appendix 1, Section 3).

7.4.7 Following sonication, let the tube stand on ice for 5 min; record the start and stop times for the incubation in the Batch Record (Appendix 1, Section 3). Then vortex at maximum speed for 10 sec.

7.4.8 Move samples to RT and add 20% SDS to a final concentration of 1% (e.g., add 20 µL 20% SDS into 400 µL lysate). Record the final volume SDS added to each sample in the Sample Information Table (Appendix 1, Section 2).

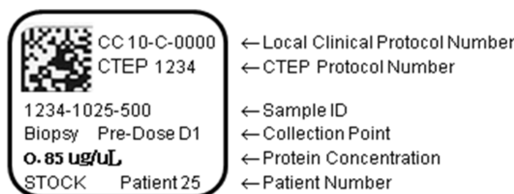
7.4.9 Vortex the sample tube at maximum speed for 10 sec and then place in a 100°C heat block or boiling water bath. Boil the specimen lysate for 5 min; record the start and stop times in the Batch Record (Appendix 1, Section 3).

7.4.10 Snap-cool specimen tube on ice after boiling, and then vortex tube at maximum speed for 10 sec.

7.5 Tumor Lysate Preparation

7.5.1 Clarify all lysates by centrifugation at 12,000 x g for 5 to 10 min at 2ºC to 8°C. Transfer the cleared lysate into a 2-mL Sarstedt tube labeled as the **stock lysate** tube (see sample label). Keep sample on ice. Discard the original tube with any precipitated material in the appropriate waste container.

- Protein concentration will be filled in using a cryogenic marker following BCA Protein Assay analysis
- Sample label for stock lysate:



7.5.2 Keep lysate on ice and perform BCA assay within 2 h.

7.5.3 If not used immediately for protein assay, snap-freeze the protein extract in liquid nitrogen or a dry ice/ethanol bath. Store the frozen samples in an 81-place freezer box, batched by patient, at -80°C until analysis.

7.6 Bicinchoninic Acid (BCA) Protein Assay

7.6.1 Perform BCA protein assay to determine stock tumor lysate protein concentration. Be sure the CEB used for the protein assay **does not** contain PI cocktail or PMSF.

You will need approximately 2 mL CEB (**without PIs)** for preparation of standards and background wells and 0.25 mL CEB (**without PIs)** per unknown sample.

7.6.2 Record the BCA Protein Assay kit lot number and date the assay is run in the Batch Record (Appendix 1, Section 4).

7.6.3 Prepare Plate Map for the Protein Assay

7.6.3.1 Use the BCA Protein Assay Plate Map (Appendix 2) for the recommended locations of the standards and unknown samples; the location of the unknown samples should match up with the sample number listed in the Sample Information Table in the Batch Record (Appendix 1, Section 2).

7.6.3.2 Each unknown sample and standard is run in duplicate. A total of 2 dilutions (1:5 and 1:10) for 12 different unknown samples can be assayed per plate.

7.6.4 Preparation of Bovine Serum Albumin (BSA) Serial Dilutions for the Standard Curve

7.6.4.1 Label seven 1.5-mL Sarstedt tubes, numbered 1 through 7, for the 2000 to 31.3 µg/mL BSA standards.

7.6.4.2 Carefully open the glass ampoule provided with the BCA Protein Assay Kit containing the 2 mg/mL BSA stock.

7.6.4.3 Using the dilution scheme below, pipette the indicated volume of CEB (**without** PIs) into each tube. Add indicated volume of BSA standard to each tube and vortex to mix. Keep samples on ice.

Tube #	Volume and Source of BSA	Volume of Diluent, CEB (without PIs)	Final BSA Conc. (µg/mL)
1 (H)	200 µL of BSA stock	0 µL	2000
2 (G)	200 µL of BSA stock	200 µL	1000
3 (F)	200 µL of tube # 2 dilution	200 µL	500
4 (E)	200 µL of tube # 3 dilution	200 µL	250
5 (D)	200 µL of tube # 4 dilution	200 µL	125
6 (C)	200 µL of tube # 5 dilution	200 µL	62.5
7 (B)	200 µL of tube # 6 dilution	200 µL	31.3

7.6.5 Preparation of Tumor Lysates for the BCA Protein Assay

7.6.5.1 For each **stock tumor lysate** to be assayed, label two1.5-mL Sarstedt tubes with the corresponding BCA sample number and the lower case letter "a," or "b" (e.g., S1a, S1b). The lower case letters represent the 2 different lysate dilutions to be assayed.

7.6.5.2 Using the clarified **stock tumor lysates** and the dilution scheme below, dilute each tumor lysate 1:5 and 1:10 with CEB (**without** PIs) in labeled 1.5-mL tubes represented by the letters a, and b, respectively. This will be sufficient volume for 25 µL of each dilution in duplicate for the BCA Protein Assay. Keep samples on ice.

Lysate Tube	Dilution	Volume and Source of Lysate	CEB (without **PIs**)
a	1:5	21 µL Tumor Lysate	84 µL
b	1:10	35 µL of tube "a"	35 µL

7.6.6 BCA Protein Assay Procedure

7.6.6.1 Label the 96-well plate and assemble all samples and standards. Pipette reagents into the plate in the following order:

WELLS	SAMPLE/REAGENT
B6 to H7	25 µL of each standard into designated duplicate wells
B2 to G5 and B8 to G11	25 µL of each tumor lysate dilution into designated duplicate wells
Remaining Wells	25 µL of CEB (**without** PIs) – Background Control

7.6.6.2 Prepare BCA Working Reagent as described in the Batch Record and record the lot number for the kit (Appendix 1, Section 1B). Pour the BCA Working Reagent into a clean multichannel pipette reservoir.

7.6.6.3 Using a multichannel pipettor, add 200 µL of the BCA Working Reagent to each well, mix by pipetting up and down being careful to prevent bubbles from forming. Change pipette tips between each 96-well plate column.

7.6.6.4 Cover plate with acetate film and incubate in a 37°C incubator for 30 min. Record the start time for the incubation in the Batch Record (Appendix 1, Section 4). At the same time, turn on the Tecan Infinite Microplate Reader so it has at least 30 min to warm up before use.

7.6.6.5 At the end of the 30 min incubation, record the end time in the Batch Record (Appendix 1, Section 4), and immediately read the plate on a Microplate Reader at 562 nm absorbance.

7.6.7 Determine Protein Concentration

7.6.7.1 Average the absorbance for the background wells A2 - A11 and each duplicate set of standards and prepare a standard curve of average absorbance (minus background) versus expected µg/mL protein. Attach a copy of the raw data and the graph of the standard curve to the Batch Record. Examples of standard curves can be obtained from the product insert.

7.6.7.2 Average the absorbance readings for each duplicate set of unknown samples, and record the average absorbance readout (minus background) for each tumor lysate dilution (a and b) in the Batch Record (Appendix 1, Section 2).

7.6.7.3 Compare the unknown lysate absorbance readouts to the standard curve to determine the protein concentration (µg/mL) for each diluted lysate sample (a and b). Record the protein concentration in µg/mL on the Sample Information Table (Appendix 1, Section 2). Divide the diluted protein concentration by 1000 and record the protein concentration in µg/µL for each.

7.6.7.4 For each unknown sample dilution (a [1:5], b [1:10]), back-calculate the protein lysate concentration for each dilution of the original lysate (multiply by 5 or 10, respectively). These values will be averaged to determine the protein concentration of the **stock tumor lysate** with the following QC criteria:

- Only average the dilutions together if the unadjusted µg/mL value of each falls within the range of the BCA assay standards (31.3 to 2000 µg/mL) and the adjusted values agree within 20% (concentration of each dilution/average concentration of all dilutions = 100% ± 20%). Record the average in the Sample Information Table in the "Avg. Conc. Corrected for Dilution" column (Appendix 1, Section 2).
- If the adjusted values do not agree within 20%, use the back-calculated lysate concentration from the dilution whose unadjusted µg/mL value falls closest to the midpoint of the standard curve (~250 µg/mL).and record it in the Sample Information Table (Appendix 1, Section 2).

7.6.7.5 Using a cryogenic marker, write the protein concentration in µg/µL on the label of the 2-mL **stock tumor lysate** tube (see sample label in SOP Step 7.5).

7.7 Quality Control (QC) Criteria for Tumor Lysates

7.7.1 Tumor lysates will be loaded based on total protein concentration in the PAR immunoassay and the final PAR concentration will be back-calculated based on the protein load and the starting tumor lysate protein concentration determined with the BCA Assay.

7.7.2 A minimal protein concentration of **0.16 µg/µL** is needed for tumor lysate to pass QC. On the Sample Information Table in the Batch Record, indicate if the samples Pass (≥ **0.16 µg/µL**) or Fail (< **0.16 µg/µL**) the protein concentration QC (Appendix 1, Section 2).

- If the stock tumor lysate concentration Fails QC (< **0.16 µg/µL**), the sample will be reported as unanalyzable in the Clinical Sample Data Report.

7.8 If the **stock tumor lysate** will be used within 8 h of lysate clarification (SOP Step 7.5.1), store on ice or at 2°C to 8°C.

7.9 **Stock tumor lysate** not used immediately for the PAR Immunoassay can be snap-frozen in liquid nitrogen or a dry ice/ethanol bath and then stored in an 81-place freezer box, batched by patient, at -80°C until analysis. Record the date and time lysates are frozen in the Batch Record (Appendix 1, Section 5).

7.10 Review and finalize the Batch Record (Appendix 1) and obtain required signatures. Document ANY and ALL deviations from this SOP in the Batch Record (Appendix 1, Section 6).

7.11 The Laboratory Director/Supervisor should review the Batch Record and print and sign their name affirming the data contained within are correct (Appendix 1, Section 7).

APPENDIX 1: 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: ____

1. Equipment and Preparation of Reagents

A. Equipment

Ultrasonic Processor: Make/Model : ______________________________

Serial #: ______________________________

Microplate Reader Make/Model: ______________________________

Serial #: ______________________________

B. Reagents

Buffers should be prepared based on volumes needed to complete all the steps. Always prepare at least 10% excess volume of buffer to ensure adequate volume to complete the study.

a. 25X Protease Inhibitor Cocktail: Dissolve one PI cocktail tablet in 2 mL ddH_20 (25X stock). The 25X stock solution is stable for 1 wk at 2°C to 8°C or 12 wk at -15°C to -25°C. If stored frozen, the material must be prepared as single-use aliquots to prevent repeat freeze-thaw.

Lot#: _____________Expiration Date: _____________

b. PMSF: Manufacturer's stock solution supplied at 100 mM. Label vial with date of receipt from manufacturer; the expiration date should be considered 6 mo after receipt.

Lot#: _____________Expiration Date: _____________

c. Cell Extraction Buffer (CEB [**without** PIs]): Manufacturer's supplied 1X solution. Keep at 2°C to 8°C.

Lot#: _____________Expiration Date: _____________

d. CEB (**with** PIs): 4.5 mL is sufficient to prepare 10 unknown samples. Keep on ice.

Reagent	Stock Concentration	Amount Needed	Final Concentration
CEB	stock	4.275 mL	N/A
PI Cocktail	25X	180 µL	1X PI Cocktail
PMSF	100 mM	45 µL	1 mM PMSF

2. Sample Information Table and BCA Protein Assay Plate Record

Sample No.	Sample ID	Vol. CEB (with PIs) (µL)	Vol. 20% SDS (µL	BCA Protein Assay							Conc. QC (Pass/Fail)*
				Tube	Dilution of Stock Lysate	Avg. Abs. (minus background)	Conc. (µg/mL)	Conc. (µg/µL)	Corrected for Dilution (µg/µL)	Avg. Conc. Corrected for Dilution (µg/µL)	
Ex.	*1234-1025-500*	*400*	*20*	*a*	*1:5*	*xxx*	*168*	*0.168*	*0.84*	*0.85*	*Pass*
				b	*1:10*	*xxx*	*85.7*	*0.086*	*0.86*		
S1				a	*1:5*						
				b	*1:10*						
S2				a	*1:5*						
				b	*1:10*						
S3				a	*1:5*						
				b	*1:10*						
S4				a	*1:5*						
				b	*1:10*						
S5				a	*1:5*						
				b	*1:10*						
S6				a	*1:5*						
				b	*1:10*						

(Table continued on next page)

* Stock lysate protein concentration, corrected for dilution, must be ≥ 0.16 µg/µL to pass QC.

BATCH RECORD: INITIALS:__________________ DATE: ___________________

Continued from previous page.

Sample No.	Sample ID	Vol. CEB (with PIs) (µL)	Vol. 20% SDS (µL	BCA Protein Assay							Conc. QC (Pass/Fail)*
				Tube	**Dilution of Stock Lysate**	**Avg. Abs.** (minus background)	**Conc. (µg/mL)**	**Conc. (µg/µL)**	**Corrected for Dilution (µg/µL)**	**Avg. Conc. Corrected for Dilution (µg/µL)**	
S7				a	*1:5*						
				b	*1:10*						
S8				a	*1:5*						
				b	*1:10*						
S9				a	*1:5*						
				b	*1:10*						
S10				a	*1:5*						
				b	*1:10*						
S11				a	*1:5*						
				b	*1:10*						
S12				a	*1:5*						
				b	*1:10*						

* Stock lysate protein concentration, corrected for dilution, must be ≥ 0.16 µg/µL to pass QC.

BATCH RECORD: INITIALS:__________________ DATE: __________________

3. Tissue Lysis

Incubate biopsies on ice for 5 min Start Time: ____:____ Stop Time: ____:____

Sonicate tissue at a setting of ________ watts for 10-15 sec; repeat 3 times on ice.

Incubate lysate on ice for 5 min Start Time: ____:____ Stop Time: ____:____

Boil lysate containing 1% SDS for 5 min Start Time: ____:____ Stop Time ____:____

4. BCA Protein Assay

BCA Working Reagent: **Prepare just before use**. Pipette 21.56 mL of Reagent A and 440 µL of Reagent B into a 50-mL polypropylene tube. Mix by inversion (the solution will turn green).

BCA Protein Assay Kit: Lot#: ________________

Date of BCA Protein Assay run ____/____/____

Incubate assay at 37°C for 30 min Start Time: ____:____ Stop Time: ____:____

Attach a copy: Raw data and the graph of the standard curve.

5. Storage of Lysates

Cell extract frozen in liquid nitrogen or dry ice/ethanol bath Date ____/____/____ Time ____:____

Sarstedt tubes placed into -80°C storage Date ____/____/____ Time ____:____

6. Notes, including any deviations from the SOP:

7. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: ____ (PRINT)

____ (SIGN)

Date: ____/________/____

APPENDIX 2: BCA PROTEIN ASSAY PLATE MAP

Plate Map for BCA protein assay set up with standards and 12 unknown sample wells (S1-S12) loaded in duplicate. Sample numbers correspond to that listed in the Sample Information in the Batch Record (Appendix 1, Section 3). The 2 different dilutions prepared for each unknown sample (1:5 and 1:10) in SOP Step 7.6.5 are represented by the letters a and b, respectively.

	1	2	3	4	5	6	7	8	9	10	11	12
A	x*	CEB (**without** PIs) – Background Control										x
B		S1a		S4a		31.25		S7a		S10a		
C		S1b		S4b		62.5		S7b		S10b		
D		S2a		S5a		125		S8a		S11a		
E		S2b		S5b		250		S8b		S11b		
F		S3a		S6a		500		S9a		S12a		
G		S3b		S6b		1000		S9b		S12b		
H	x					2000						x

B6-H7, BSA standards in duplicate

B2-G5 and B8-G11, 12 unknown samples, two dilutions each run in duplicate

Remaining wells, CEB (**without** PIs) will be loaded in all grey-colored wells in example above, but the background RLU reading can be calculated based on A3-A11.

*Readings from the 4 corner wells should not be used to determine background.