# Biopsy Specimen Processing for PAR Immunoassay

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  - Date: 2013.01.16 12:44:44 -05'00'
# Change History

<table>
<thead>
<tr>
<th>Revision</th>
<th>Approval Date</th>
<th>Description</th>
<th>Originator</th>
<th>Approval</th>
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<tr>
<td>--</td>
<td>10/16/2006</td>
<td>New Document</td>
<td>YZ</td>
<td>JJ</td>
</tr>
<tr>
<td>A</td>
<td>7/17/2007</td>
<td>Revision for Phase 1</td>
<td>YZ</td>
<td>JJ</td>
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<tr>
<td>B</td>
<td>10/14/2008</td>
<td>Merge Tissue Processing (SOP34506) with protein assay (SOP340510)</td>
<td>KG</td>
<td>JJ</td>
</tr>
<tr>
<td>C</td>
<td>12/01/2008</td>
<td>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</td>
<td>YZ</td>
<td>JJ</td>
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<tr>
<td>D</td>
<td>8/10/2009</td>
<td>Update SOP references, separated BCA sample tables to Appendix 2, added PAR IA processing flow chart</td>
<td>YAE</td>
<td>JJ</td>
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<tr>
<td>E</td>
<td>4/8/2011</td>
<td>Incorporated BCA assay into SOP work flow, incorporated Appendix 3 into Batch Record and, updated Sections 5.0 and 6.0</td>
<td>YAE</td>
<td>JJ</td>
</tr>
<tr>
<td>F</td>
<td>1/8/2013</td>
<td>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.</td>
<td>KFG, YAE</td>
<td>KFG</td>
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OVERVIEW OF PAR IMMUNOASSAY SPECIMEN PROCESSING

PBMC Processing

**SOP340503:** PBMC Collection, Preparation, and Freezing for Protein Extraction
- Collect PD blood sample from clinical site
- Purify PBMCs and determine total viable PBMCs/mL

**SOP340506:** PBMC Protein Extraction for PAR Immunoassay
- Extract protein from PBMC cell pellet to a final relative concentration of 1 x 10^7 cells/mL

**SOP340505:** Poly(ADP-ribose) (PAR) Immunoassay
- Perform ELISA with unknown samples, PAR polymer standards, and controls
- Using a Tecan Microplate reader, determine the relative signal of all samples

Tumor Biopsy Processing

**SOP340507:** Tumor Frozen Needle Biopsy Collection and Handling
- Collect fresh needle biopsy from clinical site
- Immediately place in liquid nitrogen or on dry ice/ethanol

**SOP340520:** Biopsy Specimen Processing for PAR Immunoassay
- Extract protein from tumor biopsy
- Determine total protein concentration for all samples

**SOP340530:** PAR Immunoassay Quality Control, Data Analyses, and Reporting
- Determine the PAR concentration in all samples and apply quality control standards to verify utility of assay
- Prepare a Clinical Sample Data Report for each set of unknown samples and send to the clinical protocol Principal Investigator
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

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

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 2°C 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.

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

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 two 1.5-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.

<table>
<thead>
<tr>
<th>Lysate Tube</th>
<th>Dilution</th>
<th>Volume and Source of Lysate</th>
<th>CEB (without PIs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1:5</td>
<td>21 µL Tumor Lysate</td>
<td>84 µL</td>
</tr>
<tr>
<td>b</td>
<td>1:10</td>
<td>35 µL of tube “a”</td>
<td>35 µL</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>WELLS</th>
<th>SAMPLE/REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6 to H7</td>
<td>25 µL of each standard into designated duplicate wells</td>
</tr>
<tr>
<td>B2 to G5 and B8 to G11</td>
<td>25 µL of each tumor lysate dilution into designated duplicate wells</td>
</tr>
<tr>
<td>Remaining Wells</td>
<td>25 µL of CEB (without PIs) – Background Control</td>
</tr>
</tbody>
</table>

SAIC. Frederick
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 ddH2O (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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock Concentration</th>
<th>Amount Needed</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEB stock</td>
<td></td>
<td>4.275 mL</td>
<td>N/A</td>
</tr>
<tr>
<td>PI Cocktail</td>
<td>25X</td>
<td>180 μL</td>
<td>1X PI Cocktail</td>
</tr>
<tr>
<td>PMSF 100 mM</td>
<td></td>
<td>45 μL</td>
<td>1 mM PMSF</td>
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BATCH RECORD: ___________________ INITIALS: ___________________ DATE: ___________
### Sample Information Table and BCA Protein Assay Plate Record

<table>
<thead>
<tr>
<th></th>
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(Table continued on next page)

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

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<th>Sample No.</th>
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</table>

* Stock lysate protein concentration, corrected for dilution, must be \( \geq 0.16 \, \mu g/\mu L \) to pass QC.
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.

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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.