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Doc. #:	SOP341210	Revision:	--	Effective Date:	06/08/2022

Clinical Pharmacodynamics Biomarker Program

Applied Developmental Directorate, Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research

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LHTP Approval: Ralph E. Parchment Date: _____

DCTD OD Approval: Toby Hecht Date: _____

Change History

Revision	Approval Date	Description	Originator	Approval
--	06/08/2022	New Document (Modified SOP341201)	LL, KFG, AKS	REP

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 IMMUNOASSAY SAMPLE PROCESSING

SOP340507: Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipping for PADIS, Frederick National Laboratory for Cancer Research (FNLCR) **OR** **SOP340567:** Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipment to EET Biobank	• Collect tumor needle biopsies in 1.5-mL conical tubes • Immediately place in liquid nitrogen or on dry ice/ethanol to flash freeze within 2 min of collection • Ship to biopsy processing laboratory or biorepository

SOP341210: Tumor Biopsy Protein Extraction for Immunoassays	• Extract total protein from tumor needle biopsies • Determine protein concentration • Store stock lysate or immediately proceed to immunoassay

1.0 PURPOSE

Standardize the method for preparing lysates of frozen needle tumor biopsies to enable quantification of analyte levels in biopsy specimen lysates using Luminex assay or enzyme-linked immunosorbent assay (ELISA) in pharmacodynamic (PD) studies of oncology agents.

2.0 SCOPE

This procedure applies to all personnel involved in the preparation of tumor biopsy samples for use in analysis of biopsy specimen lysates in immunoassays during clinical trials. The goal of the SOP and associated training is to ensure consistency in analyte level measurements across operators and clinical sites.

3.0 ABBREVIATIONS

BSA	=	Bovine Serum Albumin
CEB	=	Cell Extraction Buffer
CV	=	Coefficient of Variation
DCTD	=	Division of Cancer Treatment and Diagnosis
ELISA	=	Enzyme-Linked Immunosorbent Assay
IA	=	Immunoassay
LHTP	=	Laboratory of Human Toxicology and Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
PADIS	=	Pharmacodynamics Assay Development & Implementation Section
PBS	=	Phosphate Buffered Saline
PBS-Casein	=	Phosphate Buffered Saline/Casein Block and Diluent
PD	=	Pharmacodynamic
QC	=	Quality Control
SD	=	Standard Deviation
SOP	=	Standard Operating Procedure
Temp	=	Temperature
WR	=	Working Reagent

4.0 INTRODUCTION

This protocol defines the procedure for preparing biopsy specimen lysates from clinical biopsies to support pharmacodynamic evaluations of analyte levels using Luminex assay or enzyme-linked immunosorbent assay (ELISA).

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. This person oversees the personnel running SOPs within the laboratory and is responsible for ensuring the personnel are certified and have sufficient experience to 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 DCTD training on this SOP and 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.

5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented DCTD 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), Plate Map (Appendix 2), and Bradford Assay Calculations (Appendix 3) 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 records must be attached to the Batch Record in order to maintain a complete audit trail.

5.4 All 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 is being used.

6.0 CRITICAL REAGENTS, MATERIALS, AND EQUIPMENT REQUIRED

6.1 Roche PhosSTOP, phosphatase inhibitor cocktail tablets (Sigma, Cat #: 04906837001)
6.2 Roche cOmplete, mini, EDTA-free protease inhibitor cocktail tablets, EASYpack (Sigma, Cat #: 04693159001)
6.3 Cell Extraction Buffer (CEB; Invitrogen, Cat#: FNN0011)
6.4 Triton X-100, non-ionic, aqueous solution, 10% w/v, stored according to manufacturer's direction (Roche, Cat#: 11332481001)
6.5 Phosphate Buffered Saline/Casein Block and Diluent, 5X concentrate (5X PBS-Casein; BioFx, Cat#: PBSC-1000-01)
6.6 Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, Cat#: 500-0006)
6.7 Bovine Serum Albumin (BSA) standard ampoules, 2 mg/mL (Thermo Scientific, Cat#: 23209)
6.8 UltraPure DNase⁄RNase-free distilled water (e.g., Invitrogen, Cat#: 10977-015)
6.9 10X Phosphate Buffered Saline pH 7.2 (PBS; Invitrogen, Cat#: 70013-072) [Dilute 1:10 in UltraPure DNase⁄RNase-free distilled water to prepare 1X PBS for use in assay.]
6.10 Pipettors (200-1000 µL and 10-100 µL) and tips
6.11 Multichannel pipettor (5-50 µL and 30-300 µL) and tips
6.12 2.0-mL Sarstedt O-ring screw cap, skirted tubes (Sarstedt, Cat#: 72.694.006)
6.13 50-mL polypropylene tubes (e.g., Becton Dickinson, Cat#: 352098)
6.14 0.4-mL 96-well flat bottom plate, clear (Nunc, Cat#: 260836)
6.15 Kimwipes (e.g., Fischer Scientific, Cat#: 06-666A)
6.16 Printable microcentrifuge tube labels
6.17 81-place freezer storage boxes (e.g., Fisher Scientific, Cat#: 12-565-182)
6.18 PRO200 Homogenizer, 120 V (Pro Scientific, Cat#: 01-01200)
6.19 Multi-Gen 7 motor unit adapter (Pro Scientific, Cat#: 07-07200)
6.20 Multi-Gen homogenizer generator, 5 mm (Pro Scientific, Cat#: 02-05075)
6.21 Micro Dissecting Spring Scissors (Roboz Surgicals, Cat#: RS-5650)
6.22 Sorvall Fresco centrifuge, refrigerated at 2ºC to 8ºC (Fisher Scientific)
6.23 Vortex mixer, digital, 500-3000 rpm (Fisher-Scientific, Cat#: 02-215-370)
6.24 Standard orbital shaker (e.g., VWR International, Cat#: 89032-088)
6.25 Non-humidified, fixed temperature incubator able to maintain 25°C (± 3°C)
6.26 Infinite® 200 or Infinite M200 Pro Microplate Reader (Tecan US) with instrument included i-control microplate reader software (alternative: Magellan data analysis software)
6.27 Dry ice
6.28 Ice bucket
6.29 -20ºC and -80ºC freezer
6.30 4°C refrigerator
6.31 Microsoft Excel 2003, 2007, 2010, or later version
6.32 Frozen needle biopsy samples collected and handled according to SOP340507 or SOP340567 (Tumor Frozen Needle Biopsy Sample Collection and Handling SOPs)

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.

7.1.1 Tumor biopsies have limited stability at -80ºC, so biopsies should be processed within 2 wk of collection or moved to either -140°C or liquid nitrogen vapor phase storage. Process **batched** samples from a single patient together to ensure consistent sample handling.

7.1.2 Tumor biopsies must be frozen within 2 min of collection as detailed in SOP340507 or SOP340567. Phosphorylated epitopes on proteins rapidly degrade, as observed in a preclinical model evaluation of ischemia time and the impact on the levels of total and phosphorylated proteins.

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

7.3 Record model and serial numbers of the equipment to be used in the Batch Record (Appendix 1, Section 1).

7.4 Tissue Lysis

7.4.1 Fill in the Sample Information Table in the Batch Record (Appendix 1, Section 2A) with Patient/Sample ID for each biopsy to be processed. Biopsy samples are kept on dry ice and processed **individually** through the homogenization steps (SOP Steps 7.4.3-7.4.7). It is not recommended to process more than 10 samples at a time.

7.4.1.1 The Patient/Sample 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 Determine the total volume of Cell Extraction Buffer (CEB) working solution needed for all samples using the calculations in Appendix 1, Section 2B of the Batch Record. For xenograft tumor quadrant extraction, determine pre-chilled CEB volume according to Appendix 4: Processing Preclinical Tumor Quadrants.

7.4.2.1 Prepare the CEB working solution (CEB [**with** inhibitors]) by dissolving one Protease Inhibitor (PI) cocktail tablet and one PhosSTOP tablet into each 10 mL pre-chilled CEB needed.

7.4.2.2 Record the lot numbers and expiration dates for the CEB, PhosSTOP and Protease Inhibitor tablets in Appendix 1, Section 2B.

7.4.3 In addition, fill a small (50 mL) beaker with wet ice for the homogenization steps. To **one** 1.5 mL Sarstedt, conical bottomed skirted tube containing an 18 g frozen needle biopsy, add 350 µL pre-chilled CEB (**with** inhibitors). For xenograft tumor quadrant extraction, use the pre-determined pre-chilled CEB volume according to Appendix 4. Record the volume used in the Sample Information Table (Appendix 1, Section 2A).

7.4.4 Place the biopsy tube in the small beaker with wet ice, mince quickly with spring scissors multiple times and immediately homogenize the biopsy with the PRO200 homogenizer with Multi-Gen adaptor and 5 mm generator at the maximum setting (4-5) for 5-10 sec until no large pieces remain. The tube should remain on ice throughout the homogenization process. Record the actual homogenizer setting in the Batch Record (Appendix 1, Section 2C).

7.4.5 Vortex for 10 sec at maximum speed on the Digital Vortex Mixer (3000 rpm).

7.4.6 Homogenize tissue on ice again at the maximum setting (4-5) for 5-10 sec to ensure all tissue pieces are dispersed. Vortex for 10 sec at maximum speed (3,000 rpm).

7.4.7 Immediately place the homogenized biopsy sample in an ice/water bath and incubate for 60 ± 5 min at 2ºC to 8ºC on a standard orbital shaker with shake speed set at 4 (or at 200 rpm for shakers with rpm setting). Vortex samples every 20 min for 10 sec while samples are shaking. For each sample, record the start and stop time of the incubation on the shaker in the Batch Record (Appendix 1, Section 2C).

7.4.7.1 If there are additional biopsies to be homogenized, fill a 50 mL polypropylene tube with ~20 mL sterile water, immerse the end of the 5 mm grinder in the water, and run at the maximum setting (4-5) for 2-3 sec. Wipe the grinder with a clean Kimwipe and return to SOP Step 7.4.3 to process the next sample.

7.5 Tumor Lysate Preparation

7.5.1 Clarify lysates by centrifugation in a Sorvall Fresco microcentrifuge at 13,000 x g for 5 min at 2ºC to 8ºC. Transfer the cleared lysate into a prechilled 2 mL Sarstedt tube and keep lysate on ice. Discard the original tube with any precipitated material in the appropriate waste container.

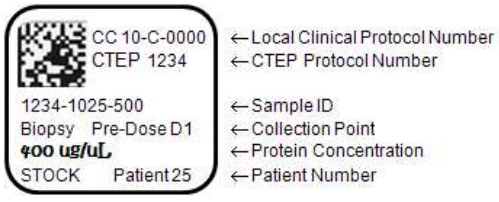
7.5.2 For each tumor lysate, place two 5 µL aliquots into labeled 1.5 mL Sarstedt tubes containing 95 µL 1X PBS (1:20 dilution) for protein analysis by Bradford Protein Assay (Section 8.0). If this is the second time a sample is being assayed, a different dilution factor may be needed. Label tube clearly with Patient/Sample ID and dilution factor.

7.5.2.1 Record the dilution used for the Bradford Protein Assay clinical sample aliquots in the Sample Information Table (Appendix 1, Section 2A).

7.5.2.2 If the Bradford Protein Assay is not performed immediately, aliquots can be snap-frozen on dry ice and stored at -80ºC for up to 5 days before analysis.

7.5.3 Aliquot the remaining **stock lysate** as 100 to 150 µL aliquots in prechilled Sarstedt tubes. Place a pre-printed specimen label (see below) on each tube.

- Protein concentration will be filled in using a cryogenic marker.
- Sample label for stock lysate:



7.6 Snap-freeze aliquots on dry ice, and store at -80ºC. Record the date and time stock lysates are frozen in the Batch Record (Appendix 1, Section 3).

8.0 BIO-RAD (BRADFORD) PROTEIN ASSAY

8.1 Prepare Plate Map for the Protein Assay

8.1.1 Generate a Plate Map of the 96-well plate (Appendix 2) to define the location of the clinical sample and BSA protein standard replicates. The sample number should match those in the Sample Information Table (Appendix 1, Section 2A). Keep a copy of the completed Plate Map with the Batch Record.

8.1.1.1 Each standard is run in duplicate.

8.1.1.2 Each clinical sample is run in duplicate; the 2 aliquots prepared in SOP Step 7.5.2 represent these replicates.

8.2 Prepare Bovine Serum Albumin (BSA) Standards

8.2.1 Label five 1.5 mL Sarstedt tubes, lettered A through E, for the Blank (A) tube and 250 to 25 µg/mL BSA standards (B-E).

8.2.2 Carefully open the glass ampoule provided with the Bradford Protein Assay kit containing the 2 mg/mL (2000 µg/mL) BSA stock and transfer to a 1.5 mL Sarstedt tube labeled as "BSA Stock."

8.2.3 Using the dilution scheme below, pipette the indicated volume of 1X PBS into each tube. Add indicated volume of BSA standard to each tube and vortex to mix. Standards are stable for 30 days at -20ºC.

Tube #	Volume and Source of BSA	Volume of Diluent, 1X PBS	Final BSA Conc. (µg/mL)
A	0 µL	500 µL	Blank
BSA Stock	1 mL BSA Stock Ampoule	N/A	2000
B	125 µL of BSA Stock	875 µL	250
C	400 µL of tube # B	600 µL	100
D	500 µL of tube # C	500 µL	50
E	500 µL of tube # D	500 µL	25

8.2.4 Keep the standards on ice.

8.3 Prepare Tumor Lysates for Bradford Protein Assay

8.3.1 If the tumor lysates are frozen, thaw on ice, vortex for 5 sec, and return to ice.

8.3.2 For each clinical sample, be sure each of the 2 diluted tumor lysate aliquots prepared in SOP Step 7.5.2 are labeled with the corresponding sample number from the Sample Information Table (Appendix 1, Section 2A). Each aliquot will be used to determine one replicate value for the clinical sample.

8.3.3 Keep samples on ice.

8.4 Bradford Protein Assay Procedure

8.4.1 Prepare 50 mL fresh Bio-Rad Protein Assay Dye working reagent (Bio-Rad WR) by mixing 40 mL water and 10 mL Dye Reagent Concentrate in a 50 mL tube.

8.4.2 Set-up and label enough 1.5 mL Sarstedt tubes to match all wells to be used in the order outlined on the Plate Map (SOP Step 8.1). Add 100 µL of each BSA standard into the appropriately labeled tube. Unknown tumor lysate samples are already in 100 µL aliquots in labeled tubes (SOP Step 7.5.2). For the blank tubes, add 100 µL 1X PBS from tube A.

8.4.3 Add 1 mL Bio-Rad WR to each tube, cap, and vortex briefly to mix.

8.4.4 Incubate samples at 25°C ± 3°C for a minimum of 5 min (maximum of 60 min). Record the date, start time, and stop time of incubation in the Batch Record (Appendix 1, Section 4).

8.4.5 Label the 96-well plate and pipette 300 µL of each sample to the appropriate wells according to the Plate Map created in SOP Step 8.1.

8.4.6 Read the plate on the Tecan Microplate Reader at 595 nm absorbance using Tecan i-Control Microplate Reader software or an equivalent spectrometer with 595 nm filter within 1 h of incubation with the Bio-Rad WR as follows:

8.4.6.1 To read the plate, click on the i-Control icon to open the software, use the pre-set settings for "Corning Costar cell culture plates 96 well", or follow manufacture's instructions if using a different spectrometer. Be sure the absorbance reading is set for 595 nm. Record the time absorbance readings are taken in the Batch Record (Appendix 1, Section 4).

8.4.6.2 The i-Control software will export the raw absorbance data for the plate into Microsoft Excel (see example in Appendix 3, Section 1).

8.5 Quality Control (QC) of Protein Assay

Note: if using the Magellan or other data analysis software for the Tecan Microplate Reader, use analysis similar to that outlined below or an equivalent data analysis process.

8.5.1 BSA Standard QC

8.5.1.1 Using the raw absorbance data, calculate the average absorbance (mean), standard deviation (SD), and percent coefficient of variation (%CV) for the blank wells and BSA standards (example in Appendix 3). Record the average absorbance of the Blank wells above the Sample Information Table (Appendix 1, Section 2A).

8.5.1.2 The mean readout of the lowest BSA standard must be > 3 SD above the mean blank readout and the highest BSA standard must be < 2.5 absorbance units or the **assay fails** and must be rerun.

8.5.1.3 Assay standard duplicates must have a % CV of < 10%. If the % CV of any assay standard is ≥ 10%, the **Assay Fails QC** and all samples must be rerun.

8.5.2 Tumor Lysate Replicate QC

8.5.2.1 Calculate the average absorbance, SD, and % CV of the unknown tumor lysate samples (example in Appendix 3) and record them in the Sample Information Table (Appendix 1, Section 2A).

8.5.2.2 Unknown tumor lysate sample duplicates must have a % CV of < 10%. If the % CV is ≥ 10%, the **Sample Fails QC;** write "Failed % CV QC" in the Sample Information Table (Appendix 1, Section 2A); the sample will need to be rerun.

8.6 Determine Protein Concentration

8.6.1 If the BSA standards passed QC, plot the BSA standard absorbance readings minus the mean of blank (Avg. Abs. Minus Blank, y-axis) versus µg/mL protein concentration (x-axis) in Excel. Plot the linear regression line for the standard curve and display the equation (**y = mx + b**) and correlation coefficient (R^2) for the line as seen in the example in Appendix 3, Section 2. Correlation coefficient (R^2) should be ≥ 0.96 for the standard curve to pass QC. If the correlation coefficient (R^2) is < 0.96, the **Standard Curve Failed QC**; write "Failed Standard Curve QC" in the Sample Information Table (Appendix 1, Section 2A); the Bradford Assay will need to be rerun.

8.6.2 Record the equation of the line, and correlation coefficient for the standard curve above the Sample Information Table (Appendix 1, Section 2A). Attach a copy of the raw data and the graph of the standard curve to the Batch Record.

8.6.3 For all unknown tumor lysate samples that passed QC, calculate the average absorbance reading minus the mean of the blank (Avg. Abs. Minus Blank) and record them in the Batch Record (Appendix 1, Section 2A).

8.6.4 Using the equation for the line of the standard curve (**y = mx + b**), determine the protein concentration for each diluted tumor lysate sample (**y** = 'Avg. Abs. Minus Blank;' **x** = 'Protein Conc. Diluted Lysate'). Record the protein concentration for each diluted sample in the Batch Record (Appendix 1, Section 2A).

8.6.5 Calculate the concentration of the stock tumor lysate ('Protein Conc. Stock Lysate') by multiplying the 'Protein Conc. Diluted Lysate' by the 'Dilution Factor' in the Batch Record (Appendix 1, Section 2A) and record the final concentration on the label of the 2 mL **stock lysate** tube.

8.7 QC Criteria for Tumor Lysates

8.7.1 Tumor lysates will be loaded based on total protein concentration in the downstream immunoassays and the final protein concentration will be back-calculated based on the protein load and the starting tumor lysate protein concentration determined with the Bradford Assay.

8.7.2 The minimal stock tumor lysate concentration needed to pass QC is determined by the minimal lysate concentration for the downstream immunoassay(s). On the Sample Information Table (Appendix 1, Section 2A), record the name of the downstream immunoassay and minimal lysate concentration requirement for the assay(s), and indicate if the samples Pass or Fail the minimal lysate protein concentration QC.

- If the stock tumor lysate concentration Fails QC for the **minimal lysate concentration**, the sample will be reported as unanalyzable in the Clinical Sample Data Report.

- If any tumor lysate sample readout falls outside the range of the standard curve (< 25 µg/mL or > 250 µg/mL), the **Sample Fails QC**; write "QC Failed" in the Sample Information Table (Appendix 1, Section 2A). The sample should be rerun with the dilution adjusted to bring it in range of the standard curve. If the original sample dilution (1:20, prepared according to Section 7.5.2) falls below the range of the assay, the sample should be rerun at a 1:10 dilution. If the sample falls above the range of the assay is should be rerun at a 1:40 dilution.

8.8 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 5).

8.9 The Laboratory Director/Supervisor should review the Batch Record and sign to affirm the data contained within are correct (Appendix 1, Section 6).

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: _____

Facility/Laboratory Running SOP: _____

Clinical Protocol Number (s): _____

Patient Number (s): _____

1. Equipment

Homogenizer	Make/Model : _____
	Serial #: _____
Standard orbital shaker	Make/Model : _____
	Serial #: _____
Microplate reader	Make/Model : _____
	Serial #: _____

2. Protein Extraction for Frozen Needle Biopsies

A. Sample Information Table

Average background absorbance (Blank): _____

Equation for the line of the standard curve: (**y = mx + b**): y = _____ x + _____

Correlation coefficient (R^2): _____

Immunoassay Name _____

Minimal Lysate Concentration for Immunoassay (µg/mL) _____

*Attach a copy of the raw data and the graph of the standard curve to the Batch Record.

No.	Patient/Sample ID	Vol. CEB (with Inhibitors) (µL)	Determine Protein Concentration: Bradford Assay							
			Avg. Abs.	SD	%CV	Avg. Abs. Minus Blank	Protein Conc. Diluted Lysate (µg/mL)	Dilution Factor	Protein Conc. Stock Lysate (µg/mL)	QC Pass/Fail
Ex:	*1234-001025-500*	*350*	*0.854*	*0.013*	*1.47*	*0.373*	*60.75*	*20*	*1215*	*Pass*
Ex:	*1234-001025-501*	*350*	*1.018*	*0.116*	*11.41*	*--*	*--*	*--*	*--*	*Fail %CV QC*
S1										
S2										
S3										
S4										
S5										
S6										
S7										
S8										
S9										
S10										

BATCH RECORD INITIALS:_____ DATE: _____

B. Calculations for Total Volume CEB Working Solution Needed

Total Vol. CEB to prepare (___ biopsies + 2) * 370 µL = _____µL

Reagent	Lot Number	Expiration Date
Cell Extraction Buffer (CEB)		
PhosSTOP, phosphatase inhibitor cocktail tablets		
cOmplete, mini, EDTA-free protease inhibitor cocktail tablets		

C. Tumor Biopsy Lysis

Homogenize frozen needle biopsies, **one at a time**, at a setting of _____. Incubate lysate at 2ºC to 8ºC on a standard orbital shaker for 60 min.

No.	Start Time	Stop Time
Ex:	*13:15*	*14:15*
S1	:	:
S2	:	:
S3	:	:
S4	:	:
S5	:	:
S6	:	:
S7	:	:
S8	:	:
S9	:	:
S10	:	:

3. Biopsy Stock Lysate Storage

Cell extract frozen on dry ice Date _____ Time ____:____

Sarstedt tubes placed into -80ºC storage Date _____ Time ____:____

4. Bradford Protein Assay

Date Bradford Protein Assay Run: _____

Incubate assay at 25°C (± 3°C) for 5 min: Start time ____:____ Stop time ____:____

Absorbance Readings: Time ____:____

5. Notes, including any deviations from the SOP:

6. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: _____

APPENDIX 2: BRADFORD PROTEIN ASSAY PLATE MAP

Setup for the Bradford Protein Assay Plate Map with standards and 10 unknown sample wells, all prepared as duplicates. Each clinical sample is diluted 1:20 for the protein assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank		S4									
B	25 µg/mL		S5									
C	50 µg/mL		S6									
D	100 µg/mL		S7									
E	250 µg/mL		S8									
F	S1		S9									
G	S2		S10									
H	S3											

A1-A2, 1X PBS

B1-E2, BSA standards

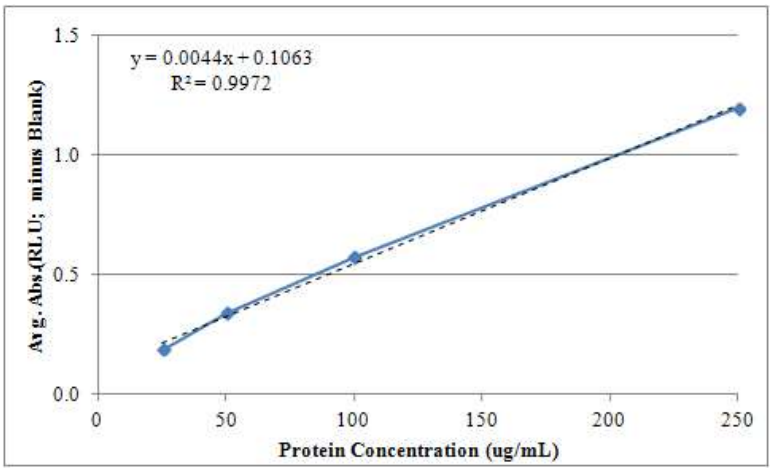
F1-H2 and A3-G4, S1-S10; 10 clinical samples, unknowns

APPENDIX 3: BRADFORD PROTEIN ASSAY DATA ANALYSES

1. Example of Standard Curve Calculations and Graph

Standard	Avg. Abs.	SD	%CV	Avg. Abs. Minus Blank*	QC
Blank	0.481	0.009	1.95	--	
25 µg/mL	0.670	0.005	0.78	0.188	Low Standard > Blank Avg. Abs. + 3 SD (= 0.508)
50 µg/mL	0.819	0.005	0.60	0.338	
100 µg/mL	1.053	0.030	2.85	0.572	
250 µg/mL	1.678	0.020	1.18	1.197	High Standard < 2.5 Abs.

*Use to plot the standard curve.



2. Example of Calculations in Sample Information Table (Appendix 1, Section 2A)

Average background absorbance (Blank): `0.481`

Equation for the line of the standard curve: (**y = mx + b**): y = `0.0044` x + `0.1063`

Correlation coefficient (R^2): `0.9972`

No.	Determine Protein Concentration: Bradford Assay							
	Avg. Abs.	SD	%CV	Avg. Abs. Minus Blank	Protein Conc. Diluted Lysate (µg/mL)	Dilution Factor	Protein Conc. Stock Lysate (µg/mL)	QC Pass/Fail
S1	0.854	0.013	1.47	0.373	60.75	20	1215	Pass
S2	0.646	0.007	0.75	0.165	13.5	20	270	Pass
S3	1.018	0.116	11.41	--	--	--	--	Failed %CV QC

APPENDIX 4: PROCESSING PRECLINICAL TUMOR QUADRANTS

For application of this procedure to xenograft tumors for preclinical applications, the use of tumor quadrants is recommended. If the xenograft tumor is not provided in a Sarstedt tube (#72.703.4160), the tumor sample should be transferred frozen (on dry ice) to the above-mentioned tube before starting extractions.

An increased volume of CEB buffer is required for xenograft tumors and should be adjusted depending on the approximate size of the tumor quadrants. The volumes below are provided as a general guideline and may need to be further optimized for each tumor model.

Approximate Tumor Quadrant Size	CEB Volume (µL)
Small (<3 mm in diameter)	370
Medium/Average (~3 – 5 mm in diameter)	500
Large (>5 mm in diameter)	750