| Title: | Biopsy Specimen Processing for MET Immunoassay | | | | Page 1 of 18 |
|---|---|---|---|---|---|
| Doc. #: | SOP341201 | Revision: | C | Effective Date: | 7/5/2016 |

# National Clinical Target Validation Laboratory (NCTVL)

## Applied Developmental Directorate, Leidos Biomedical Research, Inc.

## Frederick National Laboratory for Cancer Research

Technical Reviewer: Apurva Srivastava Date: 7/6/16

NCTVL Approval: Jiuping Ji Date: 7/6/16

IQC Approval: Katherine V. Ferry-Galow Date: 7/11/16

LHTP Approval: Ralph E. Parchment RSC Date: 8/7/2016

DCTD OD Approval: Toby Hecht TTH Date: 8/10/16

## Change History

| Revision | Approval Date | Description | Originator | Approval |
|---|---|---|---|---|
| -- | 5/12/2010 | New Document | AKS, JW | AKS |
| A | 1/9/2012 | Format for DCTD style. Define critical reagent and expand Batch Record. Assay transfer draft. | YAE | AKS |
| B | 2/17/2014 | Updated sample processing flow chart and links to current SOPs. Expanded Sample Information Table to include absorbance calculations. Define room temperature incubations throughout. | YAE | AKS |
| C | 7/5/2016 | Removed reference to pY1235 MET IA, Changes to the critical reagent list and other minor edits in preparation for community transfer. | KFG | AKS |

**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.**

# TABLE OF CONTENTS

# OVERVIEW OF MET IMMUNOASSAY SAMPLE PROCESSING

**SOP340507:**
Tumor Frozen Needle Biopsy Specimen Collection and Handling

- Collect and flash-freeze fresh tumor needle biopsies within 2 min
- Immediately place in liquid nitrogen or on dry ice/ethanol

↓

**SOP341201:** Preparation of Tumor Biopsy Lysates for MET Immunoassay(s)

- Extract protein from tumor biopsy
- Determine protein concentration
- Store stock lysate or immediately proceed to immunoassay

↓

**SOP341203:**
Intact MET Immunoassay

- Perform ELISA with clinical samples, standards, and controls
- Using Tecan Microplate reader, determine relative signal of all samples

→ Only samples with a readout of **≥ 200 fmol/mg from the Intact MET Immunoassay**, should be assessed with the phospho-MET immunoassays

↓

**SOP341206:**
Dual Phospho-Y1234/Y1235 MET Immunoassay

**SOP341205:**
Phospho-Y1356 MET Immunoassay

- Purpose: to measure increased pMET or target inhibition in overexpressed/amplified disease conditions
- Perform ELISA with clinical samples, standards, and controls
- Using Tecan Microplate reader, determine relative signal of all samples

Listed in recommended order. Mono-pMET assays is only run if dual-pMET assay is positive.

↓

**SOP341208:** MET Immunoassay(s) Quality Control, Data Analyses, and Reporting

- Determine the MET 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 of frozen needle tumor biopsies to enable quantification of MET levels with an enzyme-linked immunosorbent assay (ELISA) in pharmacodynamic (PD) studies of chemotherapeutic agents.

## 2.0 SCOPE

This procedure applies to all personnel involved in the preparation of tumor biopsy samples for use in MET immunoassays during clinical trials. The goal of the SOP and associated training is to ensure consistency in MET measurement across samples 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 |
| HGF | = | Hepatocyte Growth Factor |
| 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

The MET Immunoassays (SOP341203, SOP341205, and SOP341206) have been developed to measure the effect of chemotherapeutic agents on MET levels in tumor biopsies. An ELISA is used to first capture MET protein from total protein extracts on plates coated with a MET capture antibody reactive to the extracellular domain of MET. The captured protein is then detected using a MET detection antibody followed by a poly-HRP conjugate to allow chemiluminescent readout and quantitation of MET 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. Oversees the personnel running SOPs within the laboratory and is responsible for ensuring this person(s) is certified and has 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, Sections 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 sample table must be printed and 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 SOP version of the SOP for the assay is being used.

## 6.0 CRITICAL REAGENTS, MATERIALS, AND EQUIPMENT REQUIRED

**6.1** PhosSTOP, phosphatase inhibitor cocktail tablets (Roche, Cat#: 04906837001)

**6.2** cOmplete, mini, EDTA-free protease inhibitor cocktail tablets, EASYpack (Roche, Cat#: 04693159001)

**6.3** Pipettors (200-1000 µL and 10-100 µL) and tips

**6.4** Multichannel pipettor (5-50 µL and 30-300 µL) and tips

**6.5** 2.0-mL Sarstedt o-ring screw cap, skirted tubes (Sarstedt, Cat#: 72.694.006)

**6.6** 50-mL polypropylene tubes (e.g., Becton Dickinson, Cat#: 352098)

**6.7** 0.4-mL 96-well flat bottom plate, clear (Nunc, Cat#: 260836)

**6.8** Kimwipes (e.g., Fischer Scientific, Cat#: 06-666A)

**6.9** Printable microcentrifuge tube labels

**6.10** 81-place freezer storage boxes (e.g., Fisher Scientific, Cat#: 12-565-182)

**6.11** Dry ice

**6.12** Ice bucket

**6.13** Cell Extraction Buffer (CEB; Invitrogen, Cat#: FNN0011)

**6.14** Triton X-100, non-ionic, aqueous solution, 10% w/v, stored according to manufacturer's direction (Roche, Cat#: 11332481001)

**6.15** Phosphate Buffered Saline/Casein Block and Diluent, 5X concentrate (5X PBS-Casein; BioFx, Cat#: PBSC-1000-01)

**6.16** Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, Cat#: 500-0006)

**6.17** Bovine Serum Albumin (BSA) standard ampoules, 2 mg/mL (Thermo Scientific, Cat#: 23209)

**6.18** Cell Extraction Buffer (CEB; Invitrogen, Cat#: FNN0011)

**6.19** UltraPure DNase⁄RNase-free distilled water (e.g., Invitrogen, Cat#: 10977-015)

**6.20** 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.]

**6.21** PRO200 Homogenizer, 120 V (Pro Scientific, Cat#: 01-01200)

**6.22** Multi-Gen 7 motor unit adapter (Pro Scientific, Cat#: 07-07200)

**6.23** Multi-Gen homogenizer generator, 5 mm (Pro Scientific, Cat#: 02-05075)

**6.24** Micro Dissecting Spring Scissors (Roboz Surgicals, Cat# : RS-5650)

**6.25** Sorvall Fresco centrifuge, refrigerated (Fisher Scientific)

**6.26** Vortex mixer, digital, 500-3000 rpm (Fisher-Scientific, Cat#: 02-215-370)

**6.27** Standard orbital shaker (e.g., VWR International, Cat#: 89032-088)

**6.28** Non-humidified, fixed temperature incubator able to maintain 25°C (± 3°C)

**6.29** Infinite® 200 or Infinite M200 Pro Microplate Reader (Tecan US) with instrument included i-control microplate reader software (alternative: Magellan data analysis software)

**6.30** -20ºC and -80ºC freezer

**6.31** 4°C refrigerator

**6.32** Microsoft Excel 2003, 2007, or 2010

**6.33** Frozen needle biopsy samples processed following SOP340507 (Tumor Frozen Needle Biopsy Sample Collection and Handling)

## 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 extracted within 2 wk of collection or moved to -140°C or liquid nitrogen vapor phase storage. Process a single patient's **batched** samples to ensure consistent sample handling.

**7.1.2** Tumor biopsies must be collected and stored within 2 min of collection as detailed in SOP340507. Phosphorylated MET epitopes rapidly degrade as observed in a preclinical model evaluation of ischemia time and the impact on the level of MET and phosphorylated MET.

**7.2** Record the name and certification number 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), if applicable.

**7.3** Record equipment model and serial numbers 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 step (SOP Step 7.4.7); it is recommended to not process more than 10 samples at one 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. In addition, fill a small (50-mL) beaker with wet ice for the homogenization steps.

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** 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). Record the volume used in the Sample Information Table (Appendix 1, Section 2A).

**7.4.4** Place the biopsy tube in a 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. 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.

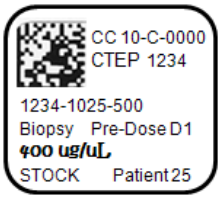
**7.5.2** For each tumor lysate, place two 5-µL aliquots into labeled 1.5-mL Sarstedt tubes containing 95 µL 1X PBS (1:20 dilution) for protein analysis by Bradford Protein Assay (SOP Step 8.0). If this is the second time a sample is being assayed, a different dilution factor may be needed. Label tube 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 d 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 two 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 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 d 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 # A | 600 µL | 100 |
| D | 500 µL of tube # B | 500 µL | 50 |
| E | 500 µL of tube # C | 500 µL | 25 |

**8.2.4** Keep samples 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 two diluted tumor lysate aliquots prepared in SOP Step 7.5.3 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.

**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 4A).

**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 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" and be sure the absorbance reading is set for 595 nm. Record the time absorbance readings are taken in the Batch Record (Appendix 1, Section 4A).

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.

#### 8.5.1 BSA Standard QC

8.5.1.1 Using the Tecan exported 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 > 3SD 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 for the line (**y = mx + b**) and correlation coefficient ($R^2$) as seen in the example in Appendix 3, Section 2.

**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 and record the concentration of the stock tumor lysate ('Protein Conc. Stock Lysate') by multiplying by the 'Protein Conc. Diluted Lysate' by the 'Dilution Factor' in the Batch Record (Appendix 1, Section 2A) and 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 MET Immunoassays and the final MET 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** A minimal stock tumor lysate concentration of **250 µg/mL** (0.25 µg/µL) is needed to pass QC. On the Sample Information Table in the Batch Record, indicate if the samples Pass (≥ **250 µg/mL**) or Fail (< **250 µg/mL**) the protein concentration QC (Appendix 1, Section 3A).

- If the stock tumor lysate concentration Fails QC (< **250 µg/mL**), 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 > 2000 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 sample falls below the range of the assay it 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).

**THIS PAGE LEFT BLANK ON PURPOSE**

# 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**

| | |
|---|---|
| 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 = m**x + **b**): y = _____ x + _____

Correlation coefficient ($R^2$): _____

*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 | | | | | | | | | | |

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 duplicate wells. 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

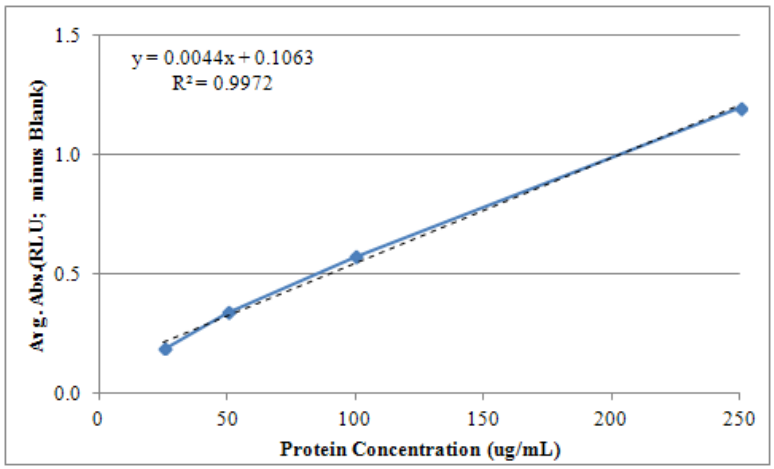
F1-H2 and A3-G4, S1-S10; 10 clinical samples, unknowns

# 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 3A)

Average background absorbance (Blank): `0.481`

Equation for the line of the standard curve: (**y = mx + b**): y = `0.0044` x + `0.1057`

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 |