

SOP340702: Biopsy Specimen Processing for TOP1 Immunoassay

Effective Date: 1/25/2013

Please check for revision status of the SOP at

<http://dctd.cancer.gov/drug-discovery-development/assays/validated-biomarker-assays>

and be sure to use the current version.

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Version History

1. Approvals

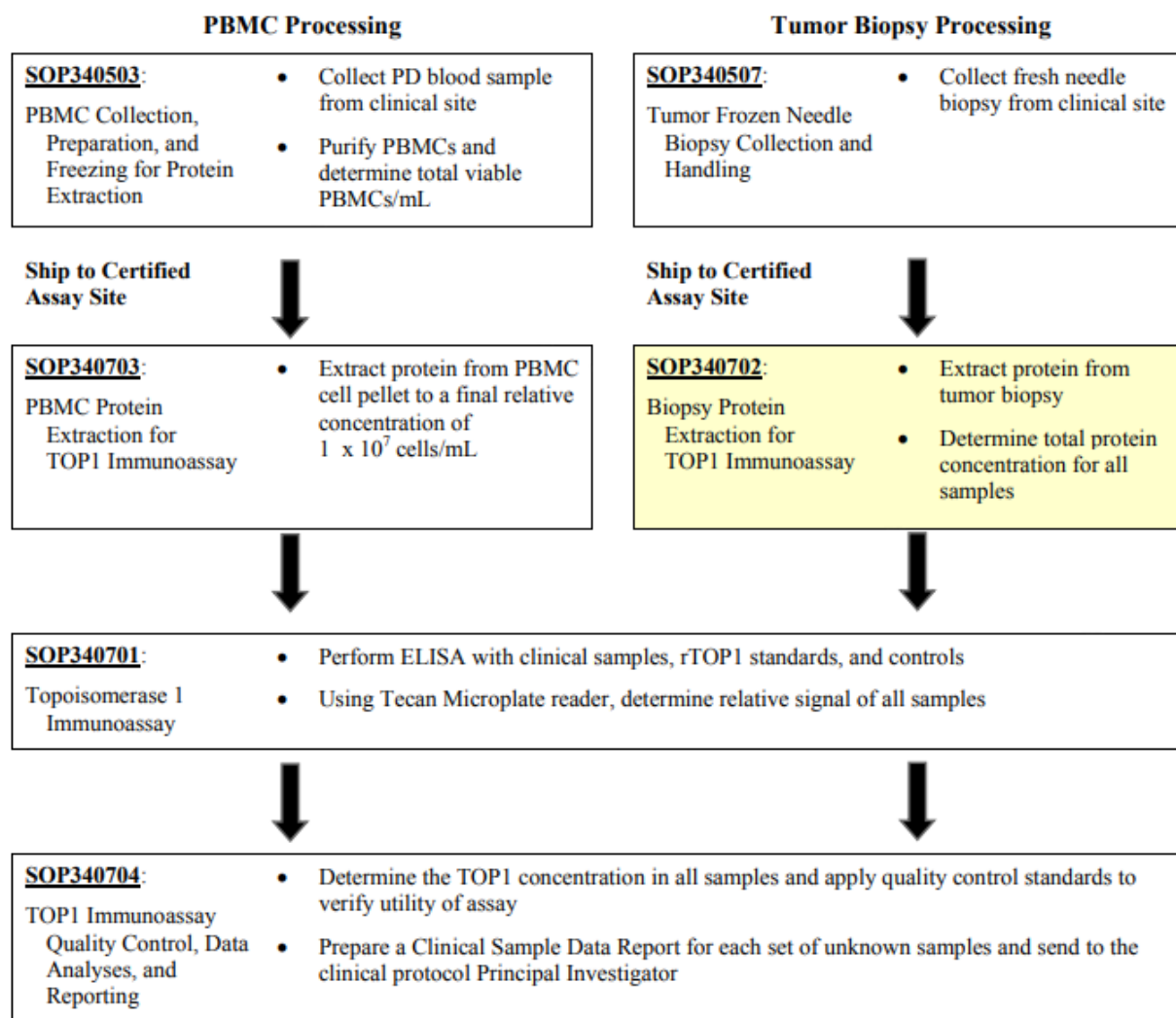
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2. Change History

Revision	Approval Date	Description	Originator	Approval
--	5/27/2011	New Document. Separate protein extraction steps from SOP340701. Create Batch Record. Updated needle biopsy processing methods and sonication times. Assay transfer complete.	YAE, TDP	JJ, RJK
A	9/24/2012	Processing steps for xenograft pieces used during assay transfer removed. Dilution of sample to 1 µg/µL removed. Minimal protein concentration QC criteria required to run the TOP1 Immunoassay defined. Requirements for digital sample tables added to SOP Step 5.3.	TDP, YZ	JJ
B	1/25/2013	Protein concentration QC criteria added. Modified extraction procedure to improve assay dilution linearity and increase maximum volume loaded per well in immunoassay.	KFG	JJ

Overview of TOP1 Immunoassay Sample Processing



1.0 PURPOSE

To standardize the method for preparing lysates of frozen needle tumor biopsies to enable quantification of topoisomerase 1 (TOP1) levels with an Enzyme-Linked ImmunoSorbent Assay (ELISA) in pharmacodynamic (PD) studies of TOP1 inhibitors.

2.0 SCOPE

This procedure applies to all personnel involved in the use of the TOP1 as a PD marker during clinical trials and in the preparation of samples for the analysis of TOP1 levels by the TOP1 Immunoassay (SOP340701). The goal of the SOP and associated training is to ensure consistency in TOP1 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
HRP	=	Horse Radish Peroxidase
IA	=	Immunoassay
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
PADIS	=	Pharmacodynamic Assay Development and Implementation Section
PBMC	=	Peripheral Blood Mononuclear Cell
PD	=	Pharmacodynamic
PI	=	Protease Inhibitor
PMSF	=	Phenylmethanesulfonyl Fluoride
RT	=	Room Temperature
SOP	=	Standard Operating Procedure
TOP1	=	Topoisomerase 1

4.0 INTRODUCTION

The TOP1 Immunoassay (SOP340701) has been developed to measure the effect of TOP1 inhibitors on TOP1 levels in a variety of biospecimen types, including PBMCs and tissue/tumor biopsies. An ELISA is used to first capture TOP1 protein from total protein extracts on plates coated with a TOP1 capture monoclonal antibody. The captured protein is then detected using a TOP1 polyclonal antibody detection antibody followed by an HRP-conjugate to allow chemiluminescent readout and quantitation of TOP1 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 oversees the personnel who follow the 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. 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. |
- 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 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 version of the SOP for the assay is being used.

6.0 MATERIALS AND EQUIPMENT REQUIRED

- 6.1 PADIS/IQC Supplied Critical Reagents
 - 6.1.1 Precellys Ceramic Bead Collection Tubes, 2.8 mm beads, 2.0-mL reinforced tubes, 50 pack (Precellys, Cat#: 03961-1-007)
- 6.2 Pipettors (200-1000 μ L, 50-200 μ L, 2-20 μ L, 0.2-2 μ L) and tips
- 6.3 250 μ L fixed volume pipettor and tips
- 6.4 Multichannel pipettor (5-50 μ L and 30-300 μ L) and tips
- 6.5 Electronic pipette
- 6.6 Disposable fine-tipped tweezers (e.g., VWR, Cat#: 83009-010)
- 6.7 Reagent reservoirs (Fisher Scientific, Cat#: 21-381-27C)
- 6.8 10- and 25-mL pipettes, sterile, individually wrapped (Fisher Scientific, Cat#: 13-675-20 and 13-668-2)
- 6.9 1.5-mL Sarstedt o-ring screw cap, conical tubes (e.g., Fisher Scientific, Cat#: 72.692.005)
- 6.10 2.0-mL Sarstedt o-ring screw cap, skirted tubes (e.g., Fisher Scientific, Cat#: 72.694.006)
- 6.11 50-mL polypropylene tubes (e.g., Becton Dickinson, Cat#: 352098)
- 6.12 Printable microcentrifuge tube labels
- 6.13 High-quality fine-tipped mincing scissors
- 6.14 Acetate microtiter plate sealers (Thermo Scientific, Cat#: 3501)
- 6.15 0.4-mL 96-well flat bottom plate, clear (Nunc, Cat#: 260836)
- 6.16 81-place freezer storage boxes (e.g., Fisher Scientific, Cat#: 12-565-182)
- 6.17 Ice bucket
- 6.18 UltraPure DNase/RNase-free distilled water (e.g., Invitrogen, Cat#: 10977-015) or Milli-Q water
- 6.19 Protease Inhibitor Cocktail (Sigma-Aldrich, Cat#: P-2714 or Roche, Cat#: 11697498001)
- 6.20 Phenylmethanesulfonyl fluoride solution, 0.1 M (PMSF; Sigma-Aldrich, Cat#: 93482-50ML-F)
- 6.21 Tris, ultra pure (e.g., MP Biomedicals, Cat#: 04819620 or 04819623)
- 6.22 Sodium chloride, ReagentPlus grade (e.g., Sigma-Aldrich, Cat#: S9625)
- 6.23 Glycerol, 100% w/v (e.g., Sigma-Aldrich, Cat#: G5516)
- 6.24 EDTA, 0.5 M, pH 8.0 (e.g., Boston BioProducts, Cat#: BM-150)
- 6.25 Magnesium chloride, anhydrous (e.g., Sigma-Aldrich, Cat#: M8266)
- 6.26 β -Glycerol phosphate disodium salt, pentahydrate (e.g., Sigma-Aldrich, Cat#: 50020)
- 6.27 Sodium fluoride, ACS grade (e.g., Sigma-Aldrich, Cat#: 201154)
- 6.28 Triton X-100, non-ionic, aqueous solution, 10% w/v, stored according to manufacturer's direction (e.g., Roche Applied Science, Cat#: 11332481001)
- 6.29 BCA Protein Assay Kit (Thermo Scientific, Cat#: 23227 or 23225)
- 6.30 Liquid nitrogen or dry ice/ethanol bath
- 6.31 Sorvall Fresco centrifuge, refrigerated (Fisher Scientific)
- 6.32 Vortex Genie 2 (Daigger, Cat#: EF3030A)
- 6.33 Precellys®24 Tissue Homogenizer (Krackeler Scientific Inc., Cat#: 224-03119.200.RD000)
- 6.34 Infinite® 200 Microplate Reader (Tecan US)
- 6.35 -20°C and -80°C freezer
- 6.36 4°C refrigerator
- 6.37 37°C incubator (e.g., Fisher Scientific, Cat#: 11-690-516D)
- 6.38 Microsoft Excel 2003, 2007, or 2010
- 6.39 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. Process a single patient's **batched** samples 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 Critical Reagents**
- 7.3.1** Record the date of receipt, lot number, and expiration date for all Critical Reagents in the Batch Record (Appendix 1, Section 1).
- 7.3.2** All Critical Reagents are to be labeled with date of receipt and stored under the specified conditions for no longer than the recommended duration. Where relevant, check that the concentrations of the provided Critical Reagents matches the concentrations cited below as some Critical Reagent dilutions may vary between lots.
- 7.3.2.1 Precellys Ceramic Bead Collection Tubes:** Store according to manufacturer's recommendations.
- 7.4** Record equipment serial numbers that will be used in the assay in the Batch Record (Appendix 1, Section 2A) and prepare the reagents outlined (Appendix 1, Section 2B).
- Note:** Do not prepare the BCA Working Reagent or CEB with PI cocktail and PMSF (**with** PIs) until noted in the SOP.
- 7.5** Fill in the Sample Information Table in the Batch Record (Appendix 1, Section 3) with the Sample ID for each biopsy. Keep all frozen needle biopsies on dry ice until ready to homogenize.
- 7.5.1** The 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).
- 7.5.2** Label sufficient Precellys ceramic bead collection for all biopsies to be processed and place on ice to chill.

Important: Work quickly, yet carefully, through SOP Step 7.6 to minimize the time between thawing of the biopsies and homogenization.

7.6 Tissue Lysis

- 7.6.1** Prepare CEB (**with** PIs) (Appendix 1, Section 2B) and store on ice.
- 7.6.2** Remove the biopsy tubes from the freezer and place on ice.
- 7.6.3** Before the biopsy is fully thawed, use a fine-tipped tweezers to transfer the tumor biopsy to the Precellys bead tube, placing it close to the bottom of the tube.
- 7.6.4** Using a fixed-volume 250- μ L pipettor, pipette 250 μ L CEB (**with** PIs) into the Precellys bead tube being sure the biopsy is submerged. Cap the bead tube and return to ice. Dispose of the tweezers in the appropriate biohazardous waste container(s).

- 7.6.5 Fill in the Sample Information Table in the Batch Record (Appendix 1, Section 3) with the CEB (**with** PIs) volume for each biopsy.
- 7.6.6 Place the tubes in the Precellys24 Tissue Homogenizer (RT) and process at a 6000 RPM for 15 sec. Wait 15 sec and repeat homogenization a second time. Record the actual homogenizer settings in the Batch Record (Appendix 1, Section 4).

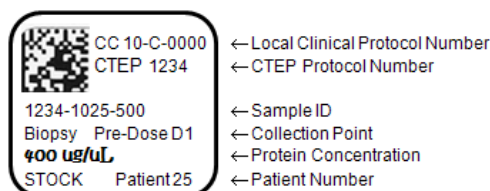
Precellys24 setting: 6000-2x15-015
PAUSE (s)

- 7.6.7 Immediately place the homogenized biopsy samples on ice and incubate for 20 min, vortexing the sample tubes at maximum speed every 5-7 min (at least 3 times). Record the time samples are placed on ice (Appendix 1, Section 4).

7.7 Tumor Lysate Preparation

- 7.7.1 Clarify all lysates by centrifugation at 13,000 x g for 10-15 min at 2°C to 8°C. Transfer the cleared lysate into a new 2-mL Sarstedt tube labeled as the **stock tumor lysate** tube (see sample label). 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**:



- 7.7.2 Keep lysate on ice and perform BCA assay within 2 h.
- 7.7.3 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 boxes, batched by patient, at -80°C until analysis.

7.8 Bicinchoninic Acid (BCA) Protein Assay

- 7.8.1 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 (**without** PIs).

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.

- 7.8.2 Record the BCA Protein Assay kit lot number and date the assay is run in the Batch Record (Appendix 1, Section 5).

7.8.3 Prepare Plate Map for the Protein Assay

- 7.8.3.1 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 3).
- 7.8.3.2 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.

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

- 7.8.4.1 Label seven 1.5-mL Sarstedt tubes, numbered 1 through 7, for the 2000 to 31.3 µg/mL BSA standards.
- 7.8.4.2 Carefully open the glass ampoule provided with the BCA Protein Assay Kit containing the 2 mg/mL BSA stock.
- 7.8.4.3 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

7.8.5 Preparation of Tumor Lysates for the BCA Protein Assay

- 7.8.5.1 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.
- 7.8.5.2 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 μ L 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 μ L Tumor Lysate	84 μ L
b	1:10	35 μ L of tube "a"	35 μ L

7.8.6 BCA Protein Assay Procedure

- 7.8.6.1 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 μ L of each standard into designated duplicate wells
B2 to G5 and B8 to G11	25 μ L of each tumor lysate dilution into designated duplicate wells
Remaining Wells	25 μ L of CEB (without PIs) – Background Control

- 7.8.6.1 Prepare BCA Working Reagent as described in the Batch Record and record the lot number for the kit (Appendix 1, Section 5). Pour the BCA Working Reagent into a clean multichannel pipette reservoir.
- 7.8.6.2 Using a multichannel pipettor, add 200 μ L 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.
- 7.8.6.3 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 5). At the same time, turn on the Tecan Infinite Microplate Reader so it has at least 30 min to warm up before use.
- 7.8.6.4 At the end of the 30 min incubation, record the end time in the Batch Record (Appendix 1, Section 5), and immediately read the plate on the Microplate Reader at 562 nm absorbance.

7.8.7 Determine Protein Concentration

- 7.8.7.1 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 $\mu\text{g/mL}$ 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.
- 7.8.7.2 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 3).
- 7.8.7.3 Compare the unknown lysate absorbance readouts to the standard curve to determine the protein concentration ($\mu\text{g/mL}$) for each diluted lysate sample (a and b). Record the protein concentration in $\mu\text{g/mL}$ on the Sample Information Table (Appendix 1, Section 3). Divide the diluted protein concentration by 1000 and record the protein concentration in $\mu\text{g}/\mu\text{L}$ for each.
- 7.8.7.4 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 $\mu\text{g/mL}$ value of each falls within the range of the BCA assay standards (31.3 to 2000 $\mu\text{g/mL}$) and the adjusted values agree within 20% (concentration of each dilution/average concentration of all dilutions = $100\% \pm 20\%$). Record the average in the Sample Information Table in the "Avg. Conc. Corrected for Dilution" column (Appendix 1, Section 3).
 - If the adjusted values do not agree within 20%, use the back-calculated lysate concentration from the dilution whose unadjusted $\mu\text{g/mL}$ value falls closest to the midpoint of the standard curve ($\sim 250 \mu\text{g/mL}$), and record it in the Sample Information Table (Appendix 1, Section 3).
- 7.8.7.5 Using a cryogenic marker, write the protein concentration in $\mu\text{g}/\mu\text{L}$ on the label of the 2-mL **stock tumor lysate** tube (see sample label in SOP Step 7.5.3).

7.9 Quality Control (QC) Criteria for Tumor Lysates

- 7.9.1 Tumor lysates will be loaded based on total protein concentration in the TOP1 immunoassay and the final TOP1 levels in each unknown sample will be back-calculated based on the μg lysate loaded in each well.
- 7.9.2 A minimal protein concentration of **0.25 $\mu\text{g}/\mu\text{L}$** is needed for tumor lysate to pass QC. On the Sample Information Table in the Batch Record, indicate if the samples Pass (\geq **0.25 $\mu\text{g}/\mu\text{L}$**) or Fail ($<$ **0.25 $\mu\text{g}/\mu\text{L}$**) the protein concentration QC (Appendix 1, Section 3).
 - If the stock tumor lysate concentration Fails QC ($<$ **0.25 $\mu\text{g}/\mu\text{L}$**), the sample should not be used for TOP1 evaluation.

- 7.10 If the **stock tumor lysate** will be used within 8 h of lysate clarification (SOP Step 7.7.1), store on ice or at 2°C to 8°C .

- 7.11** **Stock tumor lysate** not used immediately for the TOP1 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 6).
- 7.12** 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 7).
- 7.13** 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 8).

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. Critical Reagents

The critical reagents are listed below; complete the table as designated. Be sure the lot numbers on each of the reagents match those cited in the product insert accompanying the reagents.

Reagent Name	Date Received	Lot Number	Expiration Date
Precellys Ceramic Bead Collection Tubes	/ /		/ /

2. Equipment and Preparation of Reagents

A. Equipment

Tissue Homogenizer: Make/Model : _____

Serial #: _____

Microplate Reader Make/Model: _____

Serial #: _____

B. Reagents

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

- a. Cell Extraction Buffer (CEB) (**without** PIs): Prepare 500 mL of buffer at a time by adding the following reagents to 350 mL ultrapure DNase/RNase-free water. Once all reagents have been added, adjust volume to 500 mL with additional ultrapure DNase/RNase-free water. Sterile filter and store at 2°C to 8°C for no longer than 3 mo.

Reagent	Molecular Weight/ Concentration	Amount Needed	Final Concentration
Tris	121.14	3028.5 mg	50 mM Tris
NaCl	58.44	8766 mg	300 mM NaCl
Glycerol	100%	50 mL	10% Glycerol
EDTA	0.5 M	3 mL	3 mM EDTA
MgCl ₂	95.22	47.5 mg	1 mM MgCl ₂
β-Glycerol	306.11	3061.2 mg	20 mM β-Glycerol
NaF	41.99	524.75 mg	25 mM NaF

Triton X-100	10%	50 mL	1% Triton
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- b. Protease Inhibitor Cocktail Tablets: Dissolve one PI cocktail tablet in 2 mL ultrapure DNase/RNase-free water (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: _____

- c. 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: _____

- d. CEB (with PIs): Just before use, prepare 4 mL of buffer by adding 40 µL 100 mM PMSF, 160 µL 25X PI cocktail in 3.8 mL CEB (**without** PIs)(See Appendix 1, Section 2B for preparation of CEB **without** PIs). Keep on ice and discard excess buffer at the end of the experimental run.

3. Sample Information Table and BCA Protein Assay Plate Record

Sample No.	Sample ID	Vol. CEB (with PIs) (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	250	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 ≥ 0.25 μg/μL to pass QC

(Continued from previous page.)

Sample No.	Sample ID	Vol. CEB (with PIs) (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	250	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						

** Stock lysate protein concentration, corrected for dilution, must be $\geq 0.25 \mu\text{g}/\mu\text{L}$ to pass QC

4. Tissue Lysis

Homogenize tissue at a setting of _____ RPM for 15 sec; repeat 2 times.

Time samples placed on ice _____ :

5. BCA Protein Assay

BCA Working Reagent: **Prepare just before use.** Pipette 21.56 mL of Reagent A and 440 μ L 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.

6. Stock Lysate Storage

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

Date _____ / _____ / _____ Time _____ :

Sarstedt tubes placed into -80°C storage

Date _____ / _____ / _____ Time _____ :

7. Notes, including any deviations from the SOP:

8. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: _____ / _____ / _____

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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 7.8.5](#) 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.