Title:	Whole Slide Image Capture of Tumor Biopsy Slides for γH2AX, pNBS1 Immunofluorescence Assay with β-Catenin Segmentation				Page 1 of 9
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DCTD OD Approval:	Toby Hecht	Date:	Toby T. Hecht -S Digitally signed by Toby T. Hecht -S Date: 2019.05.09 08:13:13 -04'00'

Change History

Revision	Approval Date	Description	Originator	Approval
A	3/29/2019	Minor edits	KFG	REP
--	4/25/2017	New Document	KFG/DW	JJ

TABLE OF CONTENTS

OVERVIEW OF IMMUNOFLUORESCENCE ASSAY FOR BIOPSIES

SOP340507:

Tumor Frozen Needle Biopsy Specimen Collection and Handling

- Collect and freeze tumor needle biopsies for use in biomarker assays

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SOP340550:

Tumor Frozen Needle Biopsy Preparation for Pharmacodynamic Immunofluorescence Assays Utilizing Murine Testis and/or Jejunum Control Tissues

- NBF fix and paraffin embed tumor needle biopsies and positive control sample
- Section biopsies for use in IFA
- Stain slides by H&E for standard histology analysis

↓

SOP340543:

γH2AX, pNBS1 IFA Staining with β-Catenin Segmentation for Tumor Biopsy Slides

- Load biopsy and control slides into Bond-RX Processing Module
- Multiplex Bond-RX automated staining of slides with γH2AX-biotin antibody with Streptavidin-AF488, pNBS1-DIG antibody with Anti-DIG-AF647 and β-Catenin-AF546 antibody
- Stain slides with DAPI and mount cover slips

↓

Image within 72 h

SOP340544:

Whole Slide Image Capture of Tumor Biopsy Slides for γH2AX, pNBS1 IFA with β-Catenin Segmentation

- Capture images of γH2AX, pNBS1, β-Catenin-stained biopsy and control slides using Aperio ScanScope FL

↓

SOP340545:

Image Extraction and Analysis of Tumor Biopsy Slides from γH2AX, pNBS1 IFA with β-Catenin Segmentation

- Quantitate captured images of γH2AX, pNBS1, β-Catenin-stained biopsy and control slides using Definiens Architect DDR Build.

1.0 PURPOSE

To standardize immunohistochemical methods to detect and quantify histone H2AX phosphorylated at serine 139 (γH2AX) and NBS1 phosphorylated at serine 343 (pNBS1) using β-Catenin tumor segmentation for pharmacodynamic (PD) evaluation of DNA damage repair status for formalin-fixed paraffin-embedded (FFPE) tissue biopsy sections. The goal of the SOP and associated training is to ensure consistency of biomarker measurements between operators and clinical sites.

2.0 SCOPE

This procedure applies to all personnel involved in the use of the γH2AX, pNBS1 IFA with β-Catenin segmentation for tumor biopsy slides from patients participating in clinical trials. This SOP outlines the recommended procedure for whole slide image capture of stained, paraffin-embedded tumor biopsy sections.

3.0 ABBREVIATIONS

Ab	=	Antibody
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
FFPE	=	Formalin-fixed paraffin-embedded tissue
γH2AX	=	Histone H2AX Phosphorylated at Serine 139
IFA	=	Immunofluorescence Assay
LHTP	=	Laboratory of Human Toxicology & Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
pNBS1	=	NBS1 phosphorylated at serine 343
QC	=	Quality Control
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The γH2AX, pNBS1 IFA with β-Catenin segmentation is an immunohistochemistry-based staining assay developed to quantify γH2AX and pNBS1 using β-Catenin staining. Staining of β-Catenin provides tumor area masking and is used, together with pathologist annotation, to define the areas in which γH2AX and pNBS1 are quantitated.

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 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 for this SOP should be well versed and comfortable with the operation of the Aperio ScanScope FL image capture system.

5.3 The Certified Assay Operator responsible for conducting the assay is to follow this SOP with associated addendum 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.***

5.4 All responsible personnel are to check the DCTD Biomarkers website (https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm) to verify that the most recent version of this SOP is being used.

6.0 MATERIALS AND EQUIPMENT REQUIRED

6.1 Aperio ScanScope FL image capture system (Leica, Biosystems)

6.2 Aperio eSlide Manager software

6.3 Aperio ImageScope software

6.4 Kimwipes (e.g., Fisher Scientific, Cat#: 06-666A)

6.5 Bond-RX stained clinical sample slides and control slides processed according to SOP340543

7.0 OPERATING PROCEDURES

7.1 The Batch Record (Appendix 1) for image capture should be completed for a single Bond-RX staining run (up to a total of 30 slides).

7.1.1 Record the Patient ID(s) and clinical protocol numbers in the Batch Record (Appendix 1).

7.2 All slides must be correctly labeled using the most up to date Barcode Template. The barcoding captures the CTEP #, Patient/Specimen ID(s), Specimen Timepoint(s), Block ID(s), Slide # and Endpoints (all relevant stains).

7.3 Protocol for Image Capture

7.3.1 On the Batch Record (Appendix 1, Section 1A) record the name of the Laboratory and Assay Operator performing the whole slide scanning using the Aperio ScanScope FL Image System, the date the slides were stained, the date the images were captured, the name of the server where the images were saved, and the last date the light was replaced on the Aperio Imaging System.

7.3.2 Turn on the Aperio Imaging System and light source. Allow lamp to warm up for 10 min before use.

7.3.3 Aperio Settings:

7.3.3.1 The slides should be scanned using Aperio at 20x image magnification.

7.3.3.2 The approximate times for the Aperio scan exposures for this assay are as follows:

- DAPI 0.025 ms
- FITC 0.125 ms; (γH2AX-Biotin/SA-AF488)
- TRITC 0.250 ms; (β-Cat-AF546)
- Cy5 2.000 ms; (pNBS1-DIG/DIG-AF647)

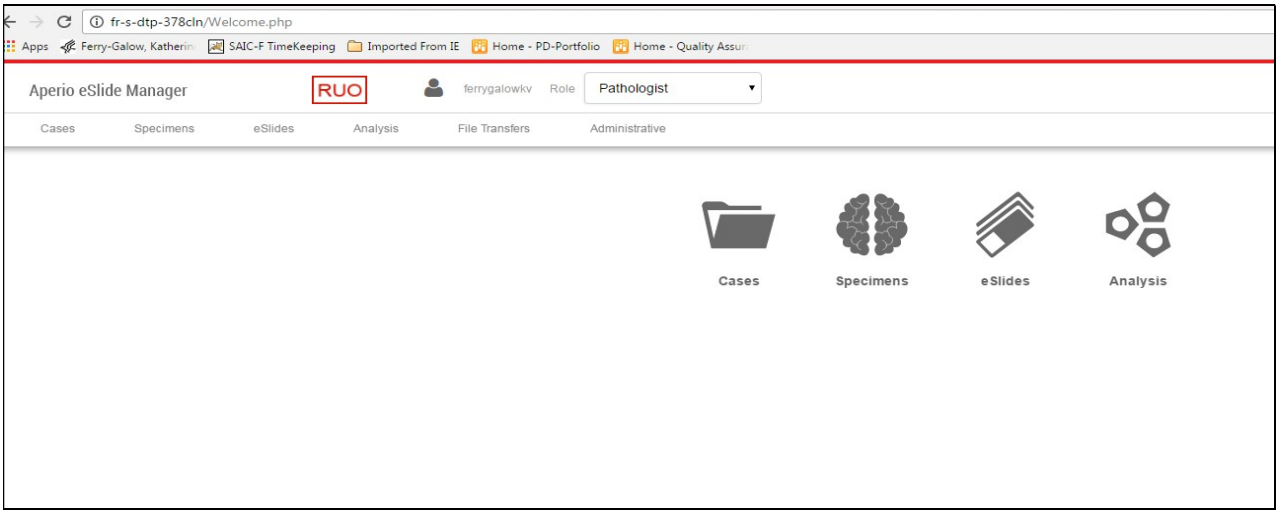
7.3.3.3 Check the γH2AX, pNBS1 and β-Catenin staining on the control slides for appropriate signal and to ensure that the staining is not overexposed. Adjust the exposure times so that the fluorescent intensity is below saturation levels.

7.3.3.4 Adjust the exposure for the DAPI channel (in milliseconds, ms) for optimal image capture. Exposures should be adjusted to minimize the underexposed nuclei while preventing appearance of overexposed nuclei to give an accurate representation of the sizes of the nuclei. Overexposure of the signal will have a "swelling" effect on the area, giving a larger "false-positive" area .

7.3.3.5 Record the adjusted Aperio scan exposure times used for each channel in Appendix 1, Section 1B.

7.3.3.6 There are 5 slide slots within the Aperio instrument. All the clinical specimens and control slides from the same staining run should be scanned in the Aperio instrument under the same acquisition parameters.

7.3.4 Storage of Images for clinical samples for NCTVL at FNLCR is located on the Clinical server "fr-s-dtp-378cln" and is accessed through eSlide Manager. A screenshot of the landing page of the Clinical Server is provided below.



7.3.5 Ensure that each scanned image appears in the appropriate folder of the eSlide Manager.

7.4 Review and finalize the Batch Record and document **ANY** and **ALL** deviations from this SOP in the Batch Record (Appendix 1, Section 2).

7.5 The Laboratory Director/Supervisor should review and sign/date the Batch Record affirming the data contained within the reports are correct (Appendix 1, Section 3).

APPENDIX 1: BATCH RECORD

Protocol #	Specimen ID	Slide #

1. Image Capture

A. Image Capture Records

Facility/Laboratory Name: _____

Assay Operator: _____

Date of Slide Staining: _____

Date of Image Capture: _____

Date of Bulb Replacement: _____

S/N or ID for ScanScope: _____

Name of Image Server: _____

B. Image Capture Records

DAPI: _____

FITC: _____

TRITC: _____

Cy5: _____

2. Notes, including any deviations from the SOP:

3. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: _____

BATCH RECORD: **INITIALS** _____ **DATE:** _____