Title:	Whole Slide Image Capture of Tumor Biopsy Slides for EMT Panel Immunofluorescence Assay				Page 1 of 9
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Please check for revision status at
https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm
and be sure to use the current version.

Change History

Revision	Approval Date	Description	Originator	Approval
--	8/01/2017	New Document	TN/KFG	JJ
A	6/16/2020	Updated SOP304549 to SOP304550 throughout the document.	KFG/LL	

TABLE OF CONTENTS

OVERVIEW OF BIOPSY PREPARATION AND PROCESSING FOR EMT PANEL IFA

SOP340507:
Tumor Frozen Needle Biopsy Specimen Collection and Handling

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

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SOP304550:
Tumor Frozen Needle Biopsy Preparation for Pharmacodynamic Immunofluorescence Assays Utilizing Murine Testis and/or Jejunum Control Tissues

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

↘

SOP304546:
EMT Panel IFA Staining for Tumor Biopsy Slides

- Load biopsy and control slides into Bond-RX Processing Module
- Bond-RX automated staining of slides with EMT Panel Critical Reagents
- Stain slides with DAPI and mount cover slips

↓ **Image within 72 hrs**

SOP304547:
Whole Slide Image Capture of Tumor Biopsy Slides for EMT Panel IFA

- Capture images of EMT IFA-stained biopsy slides and control slides using Aperio ScanScope FL

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SOP340548:
Image Extraction and Analysis of Tumor Biopsy Slides from EMT Panel IFA

- Quantitate captured images of EMT IFA-stained biopsy slides and control slides using ImageScope and Definiens Tissue Studio analysis software

1.0 PURPOSE

To standardize an immunohistochemical method for detecting and quantifying epithelial-mesenchymal transition (EMT) markers in formalin-fixed, paraffin-embedded (FFPE) human tissue sections to support pharmacodynamic (PD) studies in clinical trials. The EMT Panel includes E-cadherin and Vimentin with β-Catenin for tumor segmentation. The goal of the SOP and associated training is to ensure consistency of measurements and to standardize the reporting of PD data for EMT IFA measurements.

2.0 SCOPE

This procedure applies to all personnel involved in the use of the EMT Panel Immunofluorescence Assay (IFA) for tumor biopsies from patients participating in clinical trials. This SOP outlines the recommended procedure for image capture of EMT-stained, paraffin-embedded tumor biopsy sections.

3.0 ABBREVIATIONS

Ab	=	Antibody
AF488	=	Alexa Fluor® 488
AF546	=	Alexa Fluor® 546
AF647	=	Alexa Fluor® 647
CTNNB1	=	β-Catenin
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
EMT	=	Epithelial to mesenchymal transition
H&E	=	Hematoxylin and Eosin
ID	=	Identification/Identifier
IFA	=	Immunofluorescence Assay
LHTP	=	Laboratory of Human Toxicology & Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
QC	=	Quality Control
ROI	=	Region of Interest
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The EMT Panel IFA was developed to evaluate and quantitate the Epithelial (E-Cadherin), Mesenchymal (Vimentin) and Transitional (colocalization of Vimentin and E-Cadherin) phenotypes in tumor tissue sections. In some cancers, high levels of Vimentin and low levels of E-Cadherin have been associated with a poor prognosis.

This assay is designed to detect, quantify, and analyze changes in the amounts of cellular markers Vimentin, E-Cadherin, and their transitional overlap in biopsied tissue in support of PD clinical trials. The tissue biopsies are collected at different time-points, usually pre-dose and post-dose. The assay detects E-Cadherin and Vimentin using monoclonal antibodies that are directly conjugated to Alexa Fluor immunostaining reporters. To define the area of the tumor tissue within which markers are quantitated, a pathologist annotates the viable tumor areas, the Regions of Interest (ROI), within each biopsy. In addition, β-catenin staining is used to better define tumor cells from surrounding normal tissues, such as stroma.

5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor — The Laboratory Director/Supervisor, directs laboratory operations, supervises technical personnel, reporting of findings, and is responsible for the proper performance of all laboratory procedures. The Laboratory Director/Supervisor 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.

Assay Operator — The Assay Operator may be a Laboratory Technician/Technologist, Research Associate, or Laboratory Scientist who has been certified through DCTD training on this SOP. The Assay Operator works under the guidance of the Laboratory Director/Supervisor. This person, in accordance with the current SOP(s), performs laboratory procedures and examinations and 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 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 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.

5.4 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 SOP340546.

7.0 OPERATING PROCEDURES

7.1 The Batch Record (Appendix 1) for image capture is 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 number 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 Aperio scan exposure time should be set ***initially*** as follows:

- DAPI 0.02 ms
- FITC 1.25 ms; (E-Cadherin AF488)
- TRITC 0.500 ms; (β-Catenin AF546)
- Cy5 1.000 ms; (Vimentin AF647)

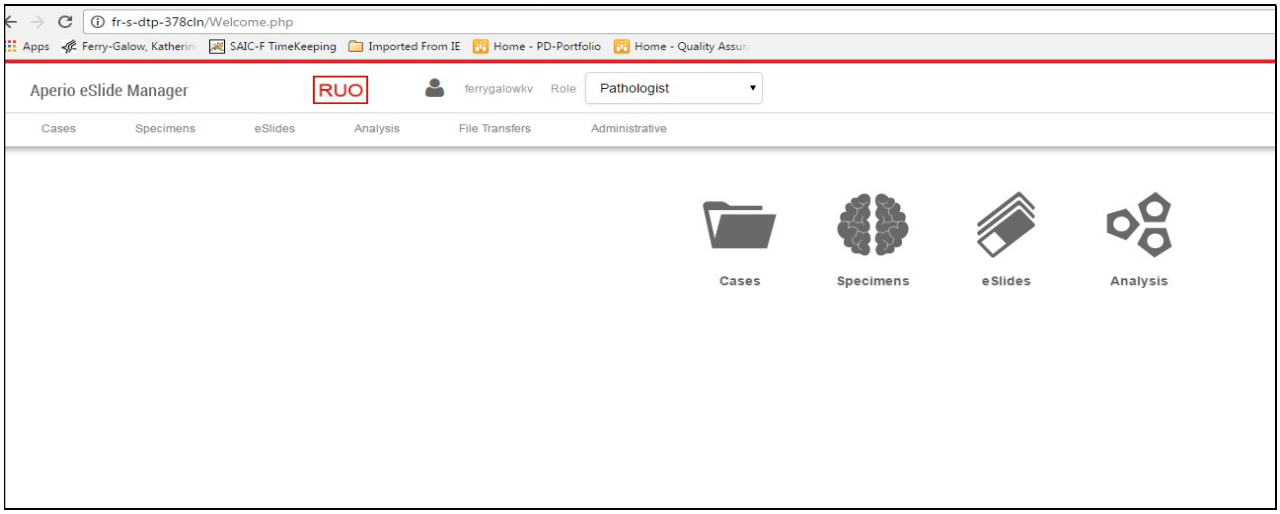
7.3.3.3 Check the HT29 tissue control for positive E-Cadherin (AF488) and β-catenin (AF546) staining to make sure that it is not overexposed. Do the same for MDA-MB-231 tissue as positive control for Vimentin staining (AF647). Adjust the exposure times accordingly so that fluorescent intensity is below 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 nuclei. Overexposure of the signal will have a "swelling" effect on the area, giving a larger "false-positive' area. For examples of images with under thresholded, over thresholded and properly thresholded nuclei, see SOP340548.

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. Be sure all the clinical specimens and control slides from the same staining run are scanned together in the Aperio instrument under the **same** acquisition parameters.

7.3.4 Images for clinical samples are stored on the Clinical server "http://fr-s-dtp-378cln/", accessed through eSlide Manager. A screenshot of the landing page of the Clinical Server is shown below:



7.3.5 Ensure that each scanned image appears in the appropriate folder of the eSlide manager and that all four channels for each image have scanned correctly.

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 #s

1. Image Capture

1. 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: _____

2. Aperio Exposure Times:

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:** _____