

LHTP003.07.23: γ H2AX, pNBS1, pKAP1 IFA Staining with β -Catenin Segmentation
for Tumor Biopsy Slides

Effective Date: 3/11/2025

Please check for revision status of the SOP at

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

TABLE OF CONTENTS

OVERVIEW OF IMMUNOFLUORESCENCE ASSAY FOR BIOPSIES.....	3
1.0 PURPOSE	4
2.0 SCOPE.....	4
3.0 ABBREVIATIONS.....	4
4.0 INTRODUCTION.....	5
5.0 ROLES AND RESPONSIBILITIES.....	5
6.0 MATERIALS AND EQUIPMENT REQUIRED	6
7.0 OPERATING PROCEDURES	8
8.0 OPTIONAL: SHIP TO CERTIFIED ASSAY SITE FOR ANALYSIS.....	19
APPENDIX 1: BATCH RECORD	20
APPENDIX 2: BOND-RX PROCESSING MODULE.....	25
APPENDIX 3: STAINED SLIDES SHIPPING MANIFEST	28

VERSION INFORMATION

1. Approvals

Technical Reviewer: Lindsay Dutko Date: _____

NCLN Approval: Li Li Date: _____

LHTP Approval: Katherine V. Ferry-Galow Date: _____

DCTD OD Approval: Toby Hecht Date: _____

2. Change History

Revision	Approval Date	Description	Originator	Approval
--	3/11/2025	New Document	AD/KFG	TH

OVERVIEW OF IMMUNOFLUORESCENCE ASSAY FOR BIOPSIES

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 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



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 control tissues
- Section biopsies for use in IFA
- Stain slides by H&E for standard histology analysis



LHTP003.07.23:

γ H2AX, pNBS1, pKAP1 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 assay specific critical reagents
- Stain slides with DAPI and mount cover slips



Image within 72 h

LHTP003.07.25:

20X Whole Slide Image Capture of IFA Tumor Biopsy Slides using a ZEISS Axioscan 7 Microscope Slide Scanner

AND

LHTP003.07.25-ADM-01

Addendum to LHTP003.07.25 for Use With γ H2AX, pNBS1, pKAP1 IFA with β -Catenin Segmentation

- Capture images of stained biopsy and control slides using a Zeiss Axioscan whole slide image scanner



LHTP003.07.26:

Whole Tissue Analysis of Tumor Biopsy Slides from γ H2AX, pNBS1, pKAP1 IFA with β -Catenin Segmentation using HALO® Image Analysis Software

- Quantitate markers from captured images of biopsy and control slides using Indica Labs HALO and HALO AI software

1.0 PURPOSE

To standardize immunohistochemical methods for staining formalin-fixed paraffin-embedded (FFPE) tissue biopsy sections to detect and quantify histone H2AX phosphorylated at serine 139 (γ H2AX), NBS1 phosphorylated at serine 343 (pNBS1) and KAP1 phosphorylated at serine 824 (pKAP1) using β -Catenin tumor segmentation for pharmacodynamic (PD) evaluation of DNA damage repair status. The goal of this 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 supporting γ H2AX, pNBS1, pKAP1 IFA staining with β -Catenin segmentation for tumor biopsy slides from patients participating in clinical trials. This SOP outlines the recommended procedure for staining slides made from paraffin-embedded tumor biopsy sections using the Leica Bond-RX™ Automatic Staining System.

3.0 ABBREVIATIONS

Ab	=	Antibody
AF	=	Alexa Fluor
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
DI	=	Deionized
DIG	=	Digoxigenin
DNP	=	Dinitrophenol
ER	=	Epitope Retrieval
FFPE	=	Formalin-fixed paraffin-embedded tissue
γ H2AX	=	Histone H2AX phosphorylated at serine 139
H&E	=	Hematoxylin and Eosin
HIER	=	Heat-Induced Epitope Retrieval
ID	=	Identification/Identifier
IFA	=	Immunofluorescence Assay
LHTP	=	Laboratory of Human Toxicology & Pharmacology
NCLN	=	National Clinical Laboratory Network
PADIS	=	Pharmacodynamics Assay Development & Implementation Section
PBS	=	Phosphate-Buffered Saline
pKAP1	=	KAP1 phosphorylated at serine 824
pNBS1	=	NBS1 phosphorylated at serine 343
IQC	=	Internal Quality Control
SA	=	Streptavidin
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The γ H2AX, pNBS1, pKAP1 IFA Staining with β -Catenin Segmentation is an immunofluorescent-based staining assay developed to quantify γ H2AX, pNBS1 and pKAP1 using β -Catenin staining to segment tumor from non-tumor to precisely limit quantitation of these DNA damage response markers to the tumor cells within a tumor biopsy section.

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 Bond-RX System.
- 5.3 Digital versions of the Bond-RX slide information and staining process including the slide event log and the first page of the slide detail log should be saved with the Batch Record in order to maintain a complete record of the procedure.
- 5.4 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*.
- 5.5 All responsible personnel are to check the DCTD Biomarkers website (<http://dctd.cancer.gov/drug-discovery-development/assays/validated-biomarker-assays>) to verify that the most recent version of this SOP is being used.

6.0 MATERIALS AND EQUIPMENT REQUIRED

- 6.1** PADIS/IQC-Supplied Critical Reagents and Control Slides
 - 6.1.1 Anti-phospho-histone H2AX (Ser139), clone JBW301, biotin conjugate (γ H2AX-Biotin); (PADIS/IQC, Part#: 30000)
 - 6.1.2 Anti-phospho-NBS1 (Ser343), clone EP178, Digoxigenin (DIG) conjugate (pNBS1-DIG); (PADIS/IQC, Part#: 30020)
 - 6.1.3 Anti-phospho-KAP1 (Ser824), clone EPR5248, Dinitrophenol (DNP) conjugate (pKAP1-DNP); (PADIS/IQC, Part#: 30051)
 - 6.1.4 Anti- β -Catenin (CTNNB1), clone E247, Alexa Fluor[®] 546 conjugate (β -Cat-AF546); (PADIS/IQC, Part#: 30025)
 - 6.1.5 Streptavidin, Alexa Fluor 750 conjugate (SA-AF750); (PADIS/IQC, Part#: 40024)
 - 6.1.6 Alexa Fluor 647 IgG Fraction Monoclonal Mouse Anti-Digoxin (DIG-AF647); (PADIS/IQC, Part#: 40008)
 - 6.1.7 Alexa Fluor 488 Anti-Dinitrophenyl KLH Rabbit Polyclonal (DNP-AF488); (PADIS/IQC, Part#: 40014)
 - 6.1.8 γ H2AX Control Slides; (PADIS/IQC, Part#: 60000)
 - 6.1.9 pNBS1/pKAP1 Control Slides; (PADIS/IQC, Part#: 60006)
- 6.2** DAPI dihydrochloride, FluoroPure[™] grade (Invitrogen, Cat#: D21490)
- 6.3** Pipettes (100-1000 μ L, 50-200 μ L, 2-20 μ L, 0.2-2 μ L) and tips
- 6.4** 50-mL polypropylene tubes (e.g., Becton Dickinson, Cat#: 352098)
- 6.5** Premium cover glasses, approx. 50 mm x 22 mm (e.g., Fisher Scientific, Cat#: 12-548-5E; Thermo Scientific, Cat#: 12440S)
- 6.6** Kimwipes (e.g., Fisher Scientific, Cat#: 06-666A)
- 6.7** Slide mailer/folder (e.g., Leica Microsystems, Cat#: 3802617)
- 6.8** Sterile-filtered, molecular biology grade deionized (DI) water (e.g., Invitrogen, Cat#: 10977-015)
- 6.9** 10X phosphate-buffered saline (PBS; e.g., Invitrogen, Cat#: 70013-073) [Dilute 1:10 in DI water to prepare 1X PBS for use in assay.]
- 6.10** Anhydrous ethanol, histology grade (e.g., Fisher Scientific, Cat#: A405-20) [Filtered using 0.22 μ m pore size before use.] ACS/USP Grade can be purchased and used without filtration. (e.g., Pharmco-AAPER, Cat#: 111000200PL05)
- 6.11** Xylene, ACS grade (e.g., EMD Millipore, Cat#: XX0055-3)
- 6.12** ProLong[®] Gold antifade reagent (Invitrogen, Cat#: P36930)
- 6.13** Bond-RX[™] Autostainer (Leica Microsystems, Cat#: 21.2701)
- 6.14** Bond Dewax Solution (Leica Microsystems, Cat#: AR9222)
- 6.15** Bond Epitope Retrieval Solution 1 (Leica Microsystems, Cat#: AR9961)
- 6.16** Bond Epitope Retrieval Solution 2 (Leica Microsystems, Cat#: AR9640)
- 6.17** Bond Open Containers 30 mL, 10 pack (Leica Microsystems, Cat#: OP309700). Alternate container sizes are listed in [Appendix 2](#).
- 6.18** Bond Research Detection Kit (Leica Microsystems, Cat#: DS9455)
- 6.19** Bond Primary Antibody Diluent (Leica Microsystems, Cat#: AR9352)
- 6.20** Normal Goat Serum Blocking Solution (e.g., Vector Laboratories, Cat#: S-1000)

-
- 6.21** Bond Universal Covertiles, 160 Pack (Leica Microsystems, Cat#: S21.4611)
 - 6.22** Bond Wash Solution 10X Concentrate (Leica Microsystems, Cat#: AR9590)
 - 6.23** Bond Universal Slide Labels and Printing Ribbon kit (Leica Microsystems, Cat#: S21.4564.A)
 - 6.24** Tissue-Tek[®] Slide Staining Dish White (Sakura, Cat#: 4457)
 - 6.25** Tissue-Tek 24-Slide Holder with Detachable Handle (Sakura, Cat#: 4465)
 - 6.26** -80°C and -20°C freezers
 - 6.27** 2°C - 8°C refrigerator
 - 6.28** Clinical slides prepared following SOP340550 with paraffin-embedded biopsy samples and control tissues on each slide

7.0 OPERATING PROCEDURES

- 7.1 Record the name of the Certified Assay Operator, the facility running the SOP and the serial number or NCI property tag number of the Bond RX unit being utilized in the Batch Record ([Appendix 1](#)).
- 7.2 If slides were shipped from a separate site, save the clinical shipping manifest for the laboratory record and maintain a copy with the Batch Record.
- 7.3 Record the unique Patient ID(s) and CTEP/Protocol #(s) for the slides being stained on the first page of the Batch Record and at the top of every subsequent page. This SOP and its associated Batch Record are sufficient for up to three Bond-RX slide trays containing up to 30 slides. A minimum of one γ H2AX and one pNBS1/pKAP1 control slide should be included with each clinical slide run. *If more than one set of patient slides were stained during a single run on the Bond-RX, all patient ID(s) & protocol #(s) must appear on all pages of the batch record.*
- 7.4 Prior to beginning the assay, ensure sufficient materials and reagents are in stock to perform the assay. All reagents are to be prepared for use in one experimental run, and only in the amounts required for the specific assay.

7.5 Critical Reagents

- 7.5.1 Record the date of receipt, lot numbers, stock reagent concentration, recommended working concentration, recommended dilution and retest dates for the critical reagents in the Batch Record ([Appendix 1, Section 1A](#)).
- 7.5.2 All Critical Reagents are to be labeled with the date of receipt and stored under the specified conditions for no longer than the recommended duration.
 - Storage conditions and retest dates for all Critical Reagents are provided on the shipping manifest that accompanies the critical reagent shipment.
 - If the Critical Reagents are purchased directly from the manufacturer, Certified Assay Sites must qualify the reagents prior to use in the Assay. Lot-to-lot differences, particularly for primary antibodies, are expected.
- 7.5.3 **Anti- γ H2AX-Biotin:** Mouse monoclonal antibody Clone JBW301 conjugated to biotin and supplied in PBS with sodium azide and glycerol. Concentration of the material as well as the recommended working concentration will be provided by lot.
- 7.5.4 **Anti-NBS1 pS343-DIG:** Rabbit monoclonal antibody Clone EP178 custom conjugated to DIG supplied in PBS with sodium azide and glycerol. Concentration of the material as well as the recommended working concentration will be provided by lot.
- 7.5.5 **Anti-KAP1 pS824-DNP:** Rabbit monoclonal antibody Clone EPR5248 custom conjugated to DNP supplied in PBS with sodium azide and glycerol. Concentration of the material as well as the recommended working concentration will be provided by lot.
- 7.5.6 **Anti- β -Catenin-AF546:** Rabbit monoclonal clone E247 custom conjugated to Alexa Fluor 546 (AF546) is supplied as a stock solution in PBS with sodium azide. The concentration of the material as well as the recommended working concentration of the material will be provided by lot.
- 7.5.7 **Streptavidin Alexa Fluor 750 (SA-AF750):** Conjugate supplied as a stock solution in PBS with BSA, sodium azide, and glycerol. The concentration of the material will be provided by lot. The optimal working concentration of SA-AF750 is 20 μ g/mL.

- 7.5.8 **Alexa Fluor 647 IgG Fraction Monoclonal Mouse Anti-Digoxin (DIG-AF647):** Conjugate supplied as a stock solution in PBS with BSA, sodium azide, and glycerol. Concentration of the material will be provided by lot. The optimal working concentration of DIG-AF647 is 17 µg/mL.
- 7.5.9 **Alexa Fluor 488 Anti-Dinitrophenyl KLH Rabbit Polyclonal (DNP-AF488):** Conjugate of rabbit polyclonal antibody to Dinitrophenyl-KLH supplied in PBS with sodium azide and glycerol. The optimal working concentration of DNP-AF488 is 20 µg/mL.
- 7.5.10 **Control Slides** should be stored in a desiccator at 2°C to 8°C away from volatile chemicals. Each clinical staining run should include one or more control slides.
- 7.5.11 **DAPI stock solution** is a 14.3 mM (5 mg/mL) solution in DI water. Aliquots can be stored at -20°C for up to 1 year. The thawed aliquots can be stored at 2°C to 8°C for up to 3 months. The solution is light sensitive, so it should be protected from light.
- 7.6** If not already done, program the following information into the Bond-RX System prior to experimental setup:
- 7.6.1 Facility or laboratory running the assay should be added to the “Researchers List”.
- 7.6.2 To enter any new antibodies and Bond Open Containers see [Appendix 2, Section 2](#) for instructions.
- 7.6.3 To enter or to verify the staining protocol, see [Appendix 2, Section 4A](#).
- 7.6.4 Verify that the HIER Protocol “HIER 10 min with ER 2” matches that listed in [Appendix 2, Section 4C](#).
- 7.6.5 If a new Research Detection Kit is being used, scan the bar code to open the **Add Reagent** dialog box. Select the name of the reagent from the **Reagent Name** drop-down list (select “Wash Buffer” for the Open Container) and in the expiration selection put a future date (suggest 1 year from current date).
- 7.7 Control and Clinical Slides**
- 7.7.1 For clinical slide runs, a minimum of one γH2AX and one pNBS1/pKAP1 control slide are recommended for each Bond-RX slide tray in use.
- 7.7.2 Clinical samples for this assay will be frozen needle biopsies collected according to SOP340507 or SOP340567 and formalin-fixed, paraffin-embedded and sectioned according to SOP340550. A minimum of two slides per biopsy tissue must be analyzed in order to report biomarker data. When possible, the slides are positioned in the slide trays so that a single patient’s slides are contained within one Bond-RX slide tray.
- 7.8 Preparation of Reagents**
- 7.8.1 During reagent preparation, record the lot number, expiration date, and preparation date as indicated in the Batch Record ([Appendix 1, Section 1B](#)). All reagents should be labeled with date of receipt and stored under the specified conditions for no longer than the recommended durations.

NOTE: Some of the following reagents may be prepared ahead of time.

7.8.2 1X Bond Wash Solution

- 7.8.2.1 Make 1 L of 1X solution by adding 100 mL Bond 10X Wash Solution to 900 mL of DI water. Mix the solution until it is homogenous and label the bottle as “1X Bond Wash Solution” with the lot number and preparation date. Store Bond 1X and 10X Wash Solutions at 2 °C to 8°C out of direct sunlight. 1X Bond Wash Solution can be used for up to 4 months.
- 7.8.2.2 When ready for use, 1X Bond Wash Solution can be poured into the bulk container marked “Wash Buffer” located within the Bond-RX Processing Module.

7.8.3 Research Detection Kit

- 7.8.3.1 Add 30 mL of 1X Bond Wash Solution to the 30-mL Open Container in the kit.

NOTE: This container is required to be loaded with the Research Detection Kit and only used for the first Bond wash.

- 7.8.4 Make sure that all required bulk reagent containers have sufficient volumes before starting the Bond-RX staining procedure. The bulk reagents containers should be at least a quarter full.

- 7.8.4.1 The bulk reagents include: 1X Bond Wash Solution, Bond Dewax solution, anhydrous ethanol, DI water, Bond Epitope Retrieval (ER) Solutions 1 and 2.

NOTE: All bulk reagents must be present in the Bond-RX regardless of if they will be used in the run. The Bond-RX will not start the run if any bulk reagents are missing or have low volume.

- 7.8.4.2 When not in use, 1X Bond Wash Solution and ER Solution containers are stored in a 2°C to 8°C refrigerator, and the other bulk reagent containers are stored in the Bond-RX bulk reagent cavity.

NOTE: Pre-warming the solutions that were stored in the refrigerator is not required; temperature does not adversely affect staining.

- 7.8.5 **Visually inspect all solutions to be used for the assay** to ensure there is no cloudiness or precipitate present. If they are cloudy or have a precipitate, discard the solutions, and clean the bottles with a mild bleach solution. Rinse the containers thoroughly with water before reuse.

7.9 **Preparation of Antibody and Ancillary Working Solutions**

- 7.9.1 Titration Containers or Open Containers for the assay working solutions are registered in the Bond-RX System as described in [Appendix 2, Section 2](#), and labeled using the Abbreviated name as follows: “DDR9”, “488/750”, “DIG-647” and “NGS”. The container size is dependent on the number of slides to be stained in a run; refer to [Appendix 2, Section 3](#) for container volumes. The container labels correspond to the steps programmed into the staining protocol ([Appendix 2, Section 4](#)).
- 7.9.2 Record the lot number and expiration date of the Bond Primary Antibody Diluent and the Bond Wash in the Batch Record ([Appendix 1, Section 1B](#)).
- 7.9.3 Perform the calculations in the Batch Record ([Appendix 1, Section 3 and Section 4](#)) to prepare the working solutions as follows:

7.9.3.1 γ H2AX/pNBS1/pKAP1/ β -Cat Primary Antibody Cocktail Solution

- The γ H2AX-Biotin /pNBS1-DIG/pKAP1-DNP/ β -Cat-AF546 Antibody Cocktail should be prepared fresh using Bond Primary Antibody Diluent. This will be used as “MARKER” in the staining protocol.
- The antibody stock concentrations and recommended dilutions for each of the four antibodies will be provided by lot.
- To ensure there is sufficient volume for all the slides to be stained, perform the calculations in the Batch Record ([Appendix 1, Section 3](#)).
- Add the calculated volume of Bond Primary Antibody Diluent to the “DDR9” container. Next, briefly warm each of the four supplied antibody critical reagent vials and then pipette the calculated volumes of each to the “DDR9” container. Record the preparation date in the Batch Record ([Appendix 1, Section 3](#)).

7.9.3.2 DNP-AF488/SA-AF750 Ancillary Working Solution

- The DNP-AF488/SA-AF750 Working Solution should be prepared fresh in 1X Bond Wash. This will be used as “DNP-AF488/SA-AF750” in the staining protocol.
- The antibody stock concentration for the DNP-AF488 Antibody and the SA-AF750 conjugate will be provided by lot. The optimal working concentration of DNP-AF488 is 20 μ g/mL and the optimal working concentration of SA-AF750 is also 20 μ g/mL.
- To be sure there is sufficient volume for all the slides to be stained, perform the calculations in the Batch Record ([Appendix 1, Section 4](#)).
- Add the calculated volume of 1X Bond Wash to the “488/750” container. Next, briefly warm the DNP-AF488 and the SA-AF750 vials and pipette the calculated volumes of each into the “488/750” container. Record the preparation date in the Batch Record ([Appendix 1, Section 4](#)).

7.9.3.3 DIG-AF647 Ancillary Working Solution

- The DIG-AF647 Working Solution should be prepared fresh in 1X Bond Wash. This will be used as “DIG-AF647” in the staining protocol.
- The antibody stock concentration for the DIG-AF647 Antibody will be provided by lot. The optimal working concentration of DIG-AF647 is 17 μ g/mL.
- To be sure there is sufficient volume for all the slides to be stained, perform the calculations in the Batch Record ([Appendix 1, Section 3](#)).
- Add the calculated volume of 1X Bond Wash to the “DIG-647” container. Next, briefly warm the DIG-AF647 and pipette the calculated volumes into the “DIG-647” container. Record the preparation date in the Batch Record ([Appendix 1, Section 3](#)).

7.9.3.4 Normal Goat Serum Block Working Solution

- The Normal Goat Serum Working Solution should be prepared fresh to a 2% final concentration in 1X Bond Wash Solution.
- To be sure there is sufficient volume for all the slides to be stained, perform the calculations in the Batch Record ([Appendix 1, Section 4](#)).
- Add the calculated volume of 1X Bond Wash to the “NGS” container. Next, briefly warm the Normal Goat Serum Block stock vial, and then pipette the calculated volumes of Normal Goat Serum into the “NGS” container. Record the preparation date in the Batch Record ([Appendix 1, Section 4](#)).

7.9.4 Always use fresh working solutions. Discard any remaining working solutions after use.

7.10 Protocol for Slide Staining in Bond-RX Processing Module

7.10.1 System Setup for Bond-RX Run

- 7.10.1.1 **Turn on** the computer and **open** the Bond software by clicking on the Bond icon, then **turn on** the Bond-RX Processing Module.
- 7.10.1.2 In the Bond software, select the **Slide Setup Screen**, and then select the **Add Study** button. In the **Add Study** window (as shown in the figure and associated table below), change the fields as suggested in the table below and then click **OK**.

Study properties

Study ID: CTEP1234-001001

Study name: 2010-10-24

Study comments:

Researcher: [dropdown menu]

[Manage researchers](#)

Study N°: 11

Dispense volume: ☐ 100 µL ☒ 150 µL

Preparation protocol: [dropdown menu]

OK Cancel

Field	Fill in
Study ID	CTEP#-Patient ID(s) (e.g., CTEP1234-001001)**
Study Name	Date of sample processing (e.g., 2010-10-24)
Study Comments	N/A
Researcher	Facility or laboratory running assay (from drop-down list)

Field	Fill in
Dispense Volume	150 µL
Preparation Protocol	Select "*----" (if off-line dewaxed) (If slides were not paraffin dipped and off-line dewaxed, use *Dewax)

** A new study will need to be added for each patient so that the slide label reflects the appropriate patient.

7.10.2 Add Slides to Bond-RX Run

7.10.2.1 While still in the **Slide Setup Screen**, click the **Add Slide** button, and in the **Add Slide** window (see image and associated table below), change the fields as shown in the table below.

2010-10-24
Study ID:
CTEP1234-001001
Researcher:

Slide ID:
Study N°:
11
Study comments:
Date created:
12/17/2018 10:19:17 AM

Add slide

PRE/C1D2 #5

Tissue type:

☒ Test tissue
☐ Negative tissue
☐ Positive tissue

Dispense volume:

☐ 100 µL
☒ 150 µL

Staining mode:

Single
Routine

Process:

☒ IHC
☐ ISH

Marker:

gH2AX_pNBS1_pKAP1_B-Cat

Protocols

Staining:

gH2AX_pNBS1_pKAP1_BCat Marker Panel

Preparation:

*- - - -

HIER:

*HIER 10 min with ER2

Enzyme:

*- - - -

Add slide
Close

Field	Fill in / Select
Slide ID	Automatically generated
Study N°	Automatically generated
Study Name	Date**
Study ID	CTEP#(s) -Patient ID(s) (**)
Comments	Sample Time-point(s) & Slide # (e.g., Pre/C1D2 #5) <u>THE SLIDE NUMBER MUST BE CHANGED FOR EACH SLIDE LABEL</u>
Tissue type	Select: Test tissue (patient slides) Positive tissue (control slide)
Dispense Volume	150 uL
Staining Mode	Single Routine
Process	IHC
Marker	Name of Marker(s) (e.g., gH2AX pNBS1 pKAP1 β-Cat)
Staining	Staining Protocol (e.g., gH2AX pNBS1 pKAP1 BCat Marker Panel)
Preparation	Select "*----" (if off-line dewaxed) (If slides were not paraffin dipped and off-line dewaxed, use *Dewax)
HIER	Select Epitope Retrieval Method (e.g., HIER 10 min with ER2)
Enzyme	"*----"

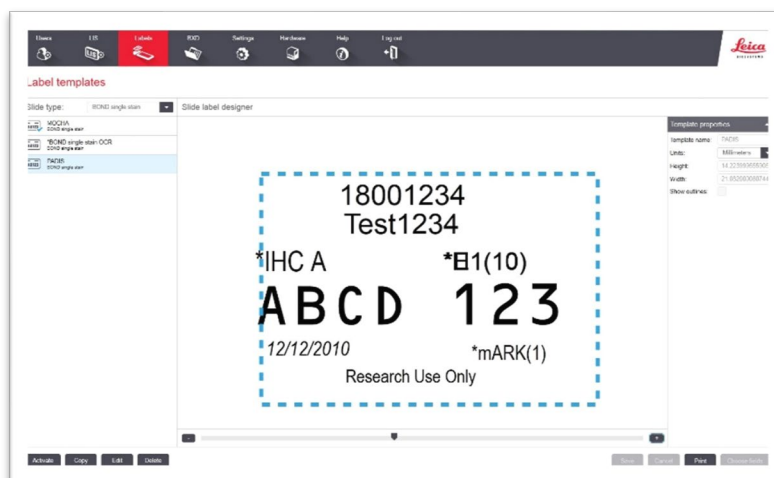
** Automatically generated from Add Study Screen

- 7.10.2.2 For each new slide, a **Bond Slide ID Number** will be assigned automatically and is listed in the upper left-hand corner of the window
- 7.10.2.3 For additional slides, click the **Add Slide** button at the bottom of the window. **Before adding slide change the slide #.**
- 7.10.2.4 Once all slides are entered, click **Close**.
- 7.10.2.5 Select the **Print Labels** button at the bottom of the screen to print the labels for the slides. Select **This Case** and click **OK**. If a label does not print correctly, right-click on the label and select **Print Label**.

7.10.3 Labels should appear with the following information shown below:

Pre/D1H2 #5
CTEP8888- 34
gH2AX/pNBS1/pKAP1/BCat
00E7 07E
8/11/2022

- 7.10.3.1 Labels may need to be modified to get all the critical information on the label and to get it in the correct order. To modify the label, click the Bond Admin icon, select Labels, and select the appropriate label template, as shown below. Click "Activate" to use this layout then exit the Bond Admin window.



7.10.3.2 Affix the printed Bond labels to the appropriate slides and make sure the labels are aligned squarely with the inside edges of the slide so that the Processing Module can scan the information.

7.10.4 Off-line Dewax of Paraffin Dipped Slides

7.10.4.1 This procedure should be followed for all Tumor Biopsy Slides and Control slides that have been dipped in paraffin to prolong stability.

- For slides that have not been dipped in paraffin, the dewaxing procedure should be carried out on the Bond-RX Automatic Staining System using the program detailed in [Appendix 2, Section 4B](#).

7.10.4.2 Prepare the reagents for the off-line dewaxing procedure in Tissue-Tek Staining dishes, and place both the clinical and control slides in a Tissue-Tek slide rack. Deparaffinize, rehydrate, and rinse slides as follows:

Number of Containers	Volume and Reagent	Incubation Time
4	200 mL Xylene	10 min each
4	200 mL Anhydrous ethanol	3 min each
3	200 mL 95% Ethanol	3 min each
3	200 mL DI water	2 min each
1	1X Bond Wash Buffer	Final wash

7.10.4.3 Record the time of initiation of the dewaxing procedure, check off the appropriate box to acknowledge the completion of each incubation step, and record the time the slide rack is placed in the Bond Wash Buffer in the Batch Record ([Appendix 1, Section 2](#)).

- 7.10.5 Remove one slide from the Bond Wash and place it in the appropriate position on a Bond Slide Tray (a minimum of one γ H2AX and one pNBS1/pKAP1 control slide are required for each Bond-RX run and will normally be placed in the last two positions of each slide tray in use). Hold a covertile at about a 20° angle above the slide, placing the wicking end of the covertile on the bottom of the frosted end of the slide.
 - 7.10.5.1 Using a transfer pipette gently apply 1X Bond Wash to the tip of the covertile and continue flush while carefully lowering the covertile onto the slide.
 - 7.10.5.2 If bubbles are introduced, remove covertile, and repeat application with a fresh covertile.
 - 7.10.5.3 Obtain next slide from the Tissue-Tek container of Bond Wash, place onto the Bond Slide Tray and repeat above covertile application process.
 - 7.10.5.4 Load Bond Slide Trays into the Bond-RX processing modules until the trays lock.
- 7.10.6 Add and Load Reagents for Bond-RX Run
 - 7.10.6.1 Go back to the Bond main menu and select the Reagent icon. Using the hand-held scanner, scan the Research Detection Kit and antibody working solution containers to enter them into the Processing Module software inventory list.
 - If you are using an Open Container or Research Detection Kit that is already in the Reagent list, after scanning the container/vial the Bond-RX interface will report the remaining volume (inventory) in that container. If there is **sufficient volume** for your current run, proceed to the next step. Otherwise, see below.
 - If the remaining volume is not sufficient, click “**Refill**” in the pop-up window before placing the containers in the Processing Module.

NOTE: 30 mL Open Containers can only be refilled to a maximum of 40 mL volume.
 - 7.10.6.2 Place the Research Detection Kit reagent tray with the Open Containers containing “Wash Buffer” and the “ γ H2AX_pNBS1_pKAP1 β -Cat”, “DNP-AF488/SA-AF750”, “DIG-AF647” and “Normal Goat Serum” working solutions into the reagent tray slot at the front of the machine and lock into position; the Wash Buffer Container needs to be placed into the first position of the reagent tray. The Processing Module will scan the reagent container bar codes to verify loading.
 - 7.10.6.3 For each loaded tray in the processing module press the **Load/Unload** button on the front of Bond RX below each tray slot to initiate scanning of the slide labels. (Tray numbering – e.g., tray 3 will load into the Processing Module closest to the reagent trays.)

NOTE: Once slides are loaded into the Processing Module, the staining procedure needs to be started within 15 min or new slide labels will need to be assigned.
 - 7.10.6.4 Once scanned, go to the computer screen, and ensure that all the labels were read correctly. If a slide label was not read correctly, right-click the corresponding slide and manually select the **Bond Slide ID** in the window.

- 7.10.7 Once all slides and reagent containers have been scanned, the **Play** button (triangle) will activate on the **System Status Screen**. Click the **Play** button on the screen to start processing the slides. **Note:** If the **Play** button does not light up, recheck that all trays are loaded correctly and that all containers have been scanned in. An error message will be displayed on the screen. Right-click on the error message and investigate as necessary.

NOTE: If the Bond Universal Covertiles are sticking to the slides during the staining procedure (they normally slide back and forth), it is likely that there is contamination in one of the bulk reagent solutions. Discard slides and all solutions. Clean bulk reagent bottles with a mild bleach solution and then rinse thoroughly with water before reuse.

7.11 Completion of Bond-RX Staining Run

- 7.11.1 Allow the Prolong Gold Antifade Reagent to equilibrate to ambient temperature (*using a heat source to warm the vial is not recommended*). If the solution appears cloudy, discard according to your institution's safety guidelines, and retrieve a fresh vial. Prolong Gold should be discarded 6 months after opening.
- 7.11.2 Just prior to slide staining completion, prepare two 250 mL Tissue-Tek staining dishes.
- 7.11.2.1 Fill the first staining dish with 200 mL DI water only. Place a Tissue-Tek 24-slide holder into this DI H₂O staining dish.
- 7.11.2.2 In the second staining dish, prepare the DAPI Working Solution by adding 10 µL of the DAPI Stock Solution to 200 mL DI water, and mix thoroughly. Protect the solution from light by covering the entire dish with aluminum foil. Record the time of Working Solution preparation in the Batch Record ([Appendix 1, Section 5](#)).
- 7.11.3 At the completion of the Bond-RX staining run, push the **Load/Unload** Button to unlock the slide trays; remove the trays from the Processing Module.
- NOTE:** Once the slides are removed from Processing Module, protect from light.
- 7.11.4 One slide at a time, remove the Bond Universal Covertile and immediately place the slide in the 24-slide holder immersed in the DI water staining dish.
- 7.11.5 Once all the slides are immersed in the DI water containing staining dish, transfer the rack to the DAPI Working Solution staining dish.
- 7.11.6 Incubate the slides for 50 min at ambient temperature in the dark (cover entire dish with aluminum foil) and gently agitate every 15 min. Record the DAPI staining start time in the Batch Record ([Appendix 1, Section 5](#)).
- 7.11.7 During the incubation time, fill three additional 250-mL staining dishes with 200 mL DI water each.
- 7.11.8 After the 50 min DAPI incubation step, remove the slide rack from the DAPI Working Solution and place it into a staining dish containing fresh DI water for 5 min. Record the time slides are removed from the DAPI Working Solution in the Batch Record ([Appendix 1, Section 5](#)).
- 7.11.8.1 Repeat the DI water wash process two additional times using a fresh DI water staining dish. Confirm the completion of wash steps in the Batch Record ([Appendix 1, Section 5](#)).

- 7.11.9 One slide at a time:
 - 7.11.9.1 Transfer the slides to a paper towel and use a Kimwipe to wick away any residual liquid, taking care not to touch the tissue or let it dry out.
 - 7.11.9.2 Using a 1000 µL pipette, place no more than two drops of Prolong Gold Antifade Reagent onto the sections and cover with a cover slip.
- 7.11.10 Place the slides in a slide book, lying flat in a safe location. Allow the slides to cure overnight in the dark at ambient temperature.
- 7.12 Slides should be stored in the dark at 2°C to 8°C and imaged **within 72 h** after cover slipping.
- 7.13 Review and finalize the Batch Record and document **ANY** and **ALL** deviations from this SOP during the slide staining process in the Batch Record ([Appendix 1, Section 6](#)).
- 7.14 The Laboratory Director/Supervisor should review the Batch Record and sample reports and sign the Batch Record affirming the data contained within the reports are correct ([Appendix 1, Section 8](#)).
- 7.15 **Clean-up**
 - 7.15.1 If this is the last experimental run of the day, be sure to **turn off** the Bond-RX Processing Module; this will ensure the lines are cleaned at the beginning of each new day when the module is turned back on. Empty the waste containers as needed.
 - 7.15.2 Store ER Solutions and 1X Wash Solution bulk reagent bottles at 2°C to 8°C. The rest of the bulk reagent containers can remain inside the body of the Bond-RX Processing Module.
 - 7.15.3 Bond Open Containers can be rinsed and used 3 times (90 mL total) for the **same** reagent. It is recommended to always use fresh working solutions.
 - 7.15.4 Place the Bond Universal Covertiles into anhydrous ethanol for 10 min to clean. Remove from ethanol and dry with a Kimwipe for reuse. If cracked or damaged, discard.
 - 7.15.5 Make sure all Bond-RX daily maintenance procedures have been completed. For overall maintenance, clean the bulk reagent bottles with a mild bleach solution every 3-6 months, rinse thoroughly with water before reuse. Additionally, at least once per month perform Cleaning and Maintenance as outlined in the Leica Bond-RX User Manual.

8.0 OPTIONAL: SHIP TO CERTIFIED ASSAY SITE FOR ANALYSIS

If the IFA analysis will be performed at a separate certified assay site, ship the slides as stated below.

IMPORTANT: Include a copy of the Batch Record for all samples being shipped, together with the Shipping Manifest.

- 8.1** Send an e-mail to the certified assay site prior to shipping to advise recipient of scheduled shipping time. Be sure to request and receive a confirmation e-mail prior to shipping.
- 8.2** Generate a shipping list containing all the specimen records using the Shipping Manifest template as shown in [Appendix 3](#).
- 8.3** **Verify** that the contents of the package match the Shipping Manifest.
- 8.4** Print and attach the shipping address to outside of the shipping container.
- 8.5** Record the shipping date, time, tracking number, and shipping information in the Batch Record ([Appendix 1, Section 7](#)).
- 8.6** Ship the specimens **with a copy of** the Shipping Manifest and copies of the completed Batch Records for all patient specimens. Retain copies of the completed Shipping Manifest and Batch Records in your records.

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 Assay: _____

Serial or NCI Property Tag Number of Bond RX: _____

Patient ID	CTEP/ Protocol #	Slide #

1. Reagents

A. Critical Reagents

Reagent Name	Date Received/ Prepared	Lot Number	Stock Reagent Conc.	Recommended Working Conc.	Recommended Dilution	Retest Date
Anti- γ H2AX-Biotin, Part: 30000	/ /					/ /
Anti-pNBS1-DIG, Part: 30020	/ /					/ /
Anti-pKAP1-DNP, Part: 30051	/ /					/ /
Anti- β -Catenin-AF546, Part: 30025	/ /					/ /
SA-AF750, Part: 40024	/ /			20 μ g/mL		/ /
Anti-DNP-AF488, Part: 40014	/ /			20 μ g/mL		/ /
Anti-DIG-AF647, Part: 40008	/ /			17 μ g/mL		/ /
γ H2AX Control Slide, Part: 60000	/ /		N/A	N/A	N/A	/ /
pNBS1/pKAP1 Control Slide, Part: 60006	/ /		N/A	N/A	N/A	/ /
DAPI	/ /		14.3 mM (5 mg/mL)	0.25 μ g/mL	1: 20,000	/ /

Patient ID(s):
CTEP/Protocol ID(s):

B. Reagent Log

Reagent	Stock Solution		Working Solution	
	Lot#	Expiration Date	Concentration	Preparation Date
10X Bond Wash Solution		/ /	1X Solution	/ /
Bond Primary Antibody Diluent		/ /	N/A	N/A

2. Off-line Dewaxing of Paraffin Dipped Slides

Were slides dipped in paraffin to prolong stability? ☐ Yes ☐ No

A. Off-line Dewax Reagent Applications

Record the times and acknowledge the reagent applications step below:

Step	Time
Time Off-line Dewax Procedure Began	:
Four, 10 min Xylene Incubations Completed	<input type="checkbox"/>
Four, 3 min Anhydrous Ethanol Incubations Completed	<input type="checkbox"/>
Three, 3 min 95% Ethanol Incubations Completed	<input type="checkbox"/>
Three, 2 min DI H ₂ O Rinses Completed	<input type="checkbox"/>
Time Slides Placed in 1X Bond Wash Solution	:

Patient ID(s):
CTEP/Protocol ID(s):

3. Preparation of Working Solutions with Two Applications

Reagent Mixture Desc. (Container Name)	Date (Prepared)	Reagent Name	Diluent Name	A. Suggested Dilution	B. Total # slides <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> x 300 µL	C. Residual vol (µL) 300µL, 1000µL, or 1500µL (see minimum requirement in Appendix 2, Section 3)	D. Total Volume: (needed for staining) B+C (µL)	E. Vol. reagent: total volume/ dilution factor D ÷ A (µL)	F. Vol. of Diluent D – M (or R)* (µL)
DDR9		1. Anti-γH2AX (M1)	Bond Primary Antibody Diluent						D - (M1+M2+M3+M4)
		2. Anti-pNBS1-DIG (M2)							
		3. Anti-pKAP1-DNP (M3)							
		4. Anti-β-Catenin-AF546 (M4)							
DIG-647		1. Anti-DIG-AF647 (R1)	1X Bond Wash						D-R1

4. Preparation of Working Solutions with One Application

Reagent Mixture Desc.	Date (Prepared)	Reagent Name	Diluent Name	A. Suggested Dilution	B. Total # slides <div style="border: 1px solid black; width: 20px; height: 20px; display: inline-block;"></div> x 150 µL	C. Residual vol (µL) 300µL, 1000µL, or 1500µL (see minimum requirement in Appendix 2, Section 3)	D. Total Volume: (needed for staining) B+C (µL)	E. Vol. reagent: total volume/ dilution factor D ÷ A (µL)	F. Vol. of Diluent D – M (or R) (µL)
488/750		1. Anti-DNP-AF488 (R1)	1X Bond Wash						D-(R1+R2)
		2. SA-750 (R2)							
NGS		Normal Goat Serum (R1)	1X Bond Wash	1:50					D-R1

After Bond-RX™ staining run is complete, save the Run Event log and the first page of the Run Detail Log*. Maintain the documents with the Batch Record. **If there was an adverse event during the Bond-RX staining run, the entire Run Detail Log should be saved and maintained with the Batch record.*

Patient ID(s):
CTEP/Protocol ID(s):

5. Staining of Slides

A. DAPI Staining and Cover Slip Application

Just prior to staining with DAPI, prepare DAPI Working Solution by diluting 10 µL DAPI stock (5 mg/mL) with 200 mL DI water in a 250-mL staining dish. Discard excess Working Solution at end of the assay run.

	Time
Slide Trays Removed from Processing Module	:
DAPI Working Solution Added to Slides	:
DAPI Working Solution Removed	:
Three, 5 min DI Water Washes Completed	<input type="checkbox"/>
ProLong Gold Antifade Reagent with Cover Slips Added	:

Patient ID:
CTEP/Protocol ID:

6. Notes, including any deviations from the SOP:

7. Shipping to Certified Assay Site

Date and time samples shipped: _____

Tracking information: _____

Attach copy of Shipping Manifest

8. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: _____

APPENDIX 2: BOND-RX PROCESSING MODULE

1. Modifications to SOP for running a single slide tray in Bond-RX System

When using a Bond Titration Container with Insert, scan the bar code on the titration container when programming the Bond-RX System, and clearly label each container. The Bond Container Insert should be discarded after use, but the Bond Titration Container can be reused multiple times.

2. Register new antibodies and Open Containers in the Bond-RX System

- A. On the Reagent Screen, add “gH2AX_pNBS1_pKAP1_BCat”, “DNP-AF488/SA-AF750”, “DIG-AF647” and “Normal Goat Serum” to the reagent list as follows:

Field	gH2AX_pNBS1_pKAP1_BCat Panel	DNP-AF488/SA-AF750	DIG-AF647	Normal Goat Serum
Name:	<i>gH2AX_pNBS1_pKAP1_BCat</i>	<i>DNP-AF488/SA-AF750</i>	<i>DIG-AF647</i>	<i>Normal Goat Serum</i>
Abbreviated name:	<i>DDR9</i>	<i>488/750</i>	<i>DIG-647</i>	<i>NGS</i>
Type:	<i>Primary</i>	<i>Ancillary</i>	<i>Ancillary</i>	<i>Ancillary</i>
Single/double stain:	<i>Single/Sequential DS</i>	<i>----</i>	<i>----</i>	<i>----</i>
Default Staining protocol:	<i>gH2AX_pNBS1_pKAP1_BCat Marker Panel</i>	<i>----</i>	<i>----</i>	<i>----</i>
Default HIER protocol:	<i>HIER 10 min with ER2</i>	<i>----</i>	<i>----</i>	<i>----</i>
Default enzyme protocol:	<i>*- - -</i>	<i>----</i>	<i>----</i>	<i>----</i>
Preferred:	<i>Selected</i>	<i>Selected</i>	<i>Selected</i>	<i>Selected</i>

- B. Scan the new Open Container, Titration Kit Container, or Research Detection Kit Container barcodes to open the Add Reagent dialog box. Select the appropriate reagent name from the Reagent Name drop-down list and label the containers with the antibody names for easy identification. Repeat this procedure for each of the assay reagents. The containers will not need to be entered again until a new container, and therefore new barcode, is used.

3. Reagent Container Sizes and Minimum Residual Volume Requirement

Reagent Containers	Max. Vol. (mL)	Minimum Required Residual Vol. (μL)
Bond Open Containers, 30 mL (Leica Microsystems, Cat#: OP309700)	30	1500
Bond Titration Kit (Containers and Inserts; Leica Microsystems, Cat#: OPT9049)	6	300
Bond Open Containers, 7 mL (Leica Microsystems, Cat#: OP79193)	7	1000

4. Staining Protocols

Create the following staining protocol (A), “gH2AX_pNBS1_pKAP1_BCat Marker Panel”, on the Bond-RX Processing Module. Protocols B and C are pre-programmed protocols on the Bond-RX Processing Module and will be used for the gH2AX_pNBS1_pKAP1_BCat Marker Panel.

A. Staining Protocol: “gH2AX_pNBS1_pKAP1_BCat Marker Panel” (protocol entered by user)

Solution	Temperature	Time (min)*
Wash Buffer†	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Normal Goat Serum	Ambient	20
MARKER	Ambient	30
MARKER	Ambient	30
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
DNP-AF488/SA-AF750	Ambient	30
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
DIG-AF647	Ambient	30
DIG-AF647	Ambient	30
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Deionized Water	Ambient	0
Deionized Water	Ambient	0
Deionized Water	Ambient	0
Deionized Water	Ambient	0
Deionized Water	Ambient	0

*A time of zero indicates that the solution is applied, but that minimal time elapses before the next application.

† The Bond-RX Processing Module requires one established solution be used from its reagent selection list. For the Research Detection Kit, 1X Bond Wash Solution is placed into a 30-mL Open Container and is used in this protocol.

B. Preparation Protocol: “*Dewax” (using Processing Module preset protocol)

Solution	Temperature (°C)	Time
Bond Dewax Solution	72	30 sec
Bond Dewax Solution	72	0
Bond Dewax Solution	Ambient	0
100% Ethanol	Ambient	0
100% Ethanol	Ambient	0
100% Ethanol	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	5 min

C. HIER Protocol: “*HIER 10 min with ER 2 (using Processing Module preset protocol)

Solution	Temperature (°C)	Time (min)
Bond ER 2 Solution	Ambient	0
Bond ER 2 Solution	Ambient	0
Bond ER 2 Solution	100	20
Bond ER 2 Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	0
Bond Wash Solution	Ambient	3

APPENDIX 3: STAINED SLIDES SHIPPING MANIFEST

<u>Ship From:</u> Contact Name: Tel: E-mail:		Shipping Manifest		<u>Ship To:</u> Attn: Tel: E-mail:	
Shipping Date:			Carrier:		
In Package	Item No.	Patient ID	Clinical Protocol/CTEP#	Bond Slide ID	Comment
<input checked="" type="checkbox"/>	<i>Example</i>	<i>Control Lot # or Patient 54</i>	<i>CTEP 8888</i>	<i>78AD</i>	<i>Control slide or Patient slide (Pre & D1H2)</i>
<input type="checkbox"/>	1				
<input type="checkbox"/>	2				
<input type="checkbox"/>	3				
<input type="checkbox"/>	4				
<input type="checkbox"/>	5				
<input type="checkbox"/>	6				
<input type="checkbox"/>	7				
<input type="checkbox"/>	8				
<input type="checkbox"/>	9				
<input type="checkbox"/>	10				