Immunofluorescence for Ventana BenchMark

Purpose	This procedure provides instructions for using Ventana BenchMark Ultra stainer for Immunofluorescence (IF) staining. IF is a powerful method for visualizing cellular processes, conditions and structures. It is a combination of two different components: First, specific antibodies, which are used to form an immune complex to mark the desired molecules – in most cases proteins – in the cell. Second, fluorochromes, which are coupled to the immune complexes and therefore visualize the target structures during fluorescence microscopy.
Scope	Histology Technical Staff
Sample	Cryostat sections
Quality Control	Positive controls are placed on the slide with patient tissue. A negative reagent control is required for each run. See antibody validation forms for documentation of antibody validation. All control slides are reviewed each day of patient testing and documented in patient report and immuno request form. All reagents and solutions must be properly labeled, as applicable and appropriate, with the following elements: • content and quantity, concentration or titer • storage requirements • date prepared or reconstituted by laboratory • expiration date • all chemical/ solution waste is handled and disposed according to established Lab Safety guidelines

Materials	**Supplies**	**Reagents**	**Equipment**
	• Charged glass slides • Cover slips • Aqua Mount • Glass Coplin jar(s) • Slide staining/ drying racks • Secureline markers	• Acetone • Liquid Nitrogen • Ventana IF antibodies and Reaction Buffer (neg.) • O.C.T. compound • Zeus Wash solution	• Cryostat • Ventana Immunostainer

Procedure	1	Preparations: • Pre chill Acetone for at least **30 minutes** to fix the tissue slides**.** • Have a chuck with OCT in the cryostat ready to receive a specimen. • Have a small container of Liquid Nitrogen ready. • Obtain IF kit from -70 freezer (kit contains 10-12 red box slides with pre-cut control tissue), labeled the slides with patient's last name and first initial and case/ accession number. IF slide assembly and stain priorities: ○ 1- H&E-first ○ 2- IgG ○ 3- IgA ○ 4- IgM ○ 5- C3 ○ 6- C1q ○ 7- Kappa ○ 8- Lambda ○ 9- Fibrinogen ○ 10- Albumin ○ 11- Negative ○ 12- H&E-last • Use only Secureline (or alcohol resistant) markers or pencils to label the slides. ***NOTE**: If the tissue is placed in Michael's media, rinse the tissue in Zeus wash solution for 30 minutes prior to freezing.*
	2	Prepare chuck by placing enough OCT on it to cover its surface, and then plunge the chuck in liquid nitrogen for 10 -15 seconds or use the heat extractor mechanism in the cryostat.
	3	Place the tissue provided by the Pathologist on the chuck and cover the tissue with OCT and freeze either in liquid nitrogen or on the heat extractor.
	4	When the chuck is ready to cut, place it in the chuck holder and face gently until the tissue emerges.
	5	Start taking the tissue sections on the pre-cut control slides and place them directly in to the pre chilled Acetone. **Make sure to pick up at least 2 tissue sections on each slide**. Do not fix H&E slides in Acetone; stain them using the frozen H&E staining process. *NOTE: If the pre-cut control slides are not available- obtain the control tissue (tonsil) from the -70 freezer and cut the control tissue. Place both patient tissue (bottom half of the slide) and control tissue (top half of the slide) on the same slide.*
	6	Fix the slides in pre chilled Acetone for 10 minutes, and air-dry them before staining.
	7	Stain the H&E slides with Rapid H&E, and screen for the Glomeruli. If Glomeruli are present in both H&E sections proceed with IF staining. IF Glomeruli are not present in either of the H&E section, inform the case Pathologist and follow their directions.
	8	To make Ultra labels: • Go to Home screen. • Click on “protocols” button on top-right. • Click on the Panel tab (IF stains- Albumin, C1q, C3, Fibrinogen, IgA, IgG, IgM, Kappa, and Lambda are added as a panel) and add the IF panel to print list. *NOTE: Since reaction buffer and the IF antibody are the only two reagents used, negative slide staining is kept offline in reaction buffer to ensure the reaction buffer is not causing the tissue to auto-fluoresce.*
	9	After adding the protocols, click “close/print” button at bottom right.
	10	Enter accession number and patient name for each barcode and click “print”. Tear off labels- pull up.
	11	Remove paper from protective cover, fold over label and place the label squarely on slide and smooth over.

	12	In the upper left corner, click on the green reagent area then in the lower left corner choose ready.
	13	Place slides on the instrument, ensuring the slide hits all four posts, labels facing toward the center of the instrument.
	14	Remove the reagents from the refrigerator, remove the lids and place the reagents on the rack, and load the rack on the instrument carousel. Close the instrument lid.
	15	In the upper left corner of the computer screen- click on the green reagent area then in the lower left corner choose ready, and click on the running button to start the instrument.
	16	Ultra will read the slides and reagents.
	17	Protocol completion times can be viewed by clicking on the slide icon (left side of screen).
	18	Place the negative slide in reaction buffer for **10 minutes**, and rinse it with distilled water and cover slip using **Aqua Mount**.
	19	Remove slides from trays that have blinking green lights- rinse them well in reaction buffer, and rinse them in distilled water until the slides run clear.
	20	Cover slip slides using **Aqua Mount** media.
	21	Label the slides with CoPath slide labels and lay them on a slide flat.
	22	Place the slide flat in the special stain refrigerator and inform the case Pathologist.
	23	Remove the tissue from the chuck, wrap it in an aluminium foil and label with patient label. Place the labeled tissue in a freezer bag and save it in the -70 freezer.
	24	Clean the cryostat and have it ready for next case.

Authorization		**Signature**	**Date**
	Medical Director	Megan K. Dishop MD	10/30/17

Annual Review	**Designee**	**Written/Revised by:**	**Effective Date:**	**Summary of Revisions**
	Histology Supervisor	Prabha Chintapalli		Initial Version