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Part 3050 Revised January 2020

CD20, B-Cell Control Slides – Technical Memo

CONTROL SLIDES:	Part 3050A	Part 3050B
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining tonsil and negative staining kidney. Fixation: Formalin 10%, Phosphate Buffered (Part 1090). Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: CD20 quality control stained slide(s) included.

Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.

Storage: 15-30°C in a light deprived and humidity controlled environment.

Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

APPLICATION:

Newcomer Supply CD20, B-Cell Control Slides are for the positive immunohistochemical staining of normal and neoplastic B-cells, generally located in follicles of lymph nodes and tonsils. CD20 is considered to be a pan-B-cell marker, occasionally detected in T-cell malignancies and a very strong marker of mature B-cell lymphomas.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- 1. Heat dry sections in oven according to your laboratory protocol.
- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 a. See Procedure Note #1.
- 3. Proceed, if necessary, with an epitope/antigen retrieval technique approved for use in your laboratory.
- 4. Rinse in distilled water; tap off excess water.
- 5. Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 6507) to reduce reagent usage and ensure tissue coverage.
- 6. Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes.
 - a. See Procedure Note #2.
- 7. Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.

a. See Procedure Note #3.

- 8. Tap off excess buffer; apply CD20 primary antibody. Incubate at room temperature for 30 minutes.
- 9. Rinse slides in two changes of buffer.
- 10. Tap off excess buffer; apply Amplifier. Incubate for 10 minutes.
- 11. Rinse slides in two changes of buffer.
- 12. Tap off excess buffer; apply HRP Polymer. Incubate for 10 minutes.
- 13. Rinse slides in two changes of buffer.
- 14. Prepare required quantity of DAB substrate/chromogen.
- 15. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
- 16. Rinse slides in four changes of distilled water.
- 17. Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
- 18. Rinse slides in warm tap water to blue sections.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

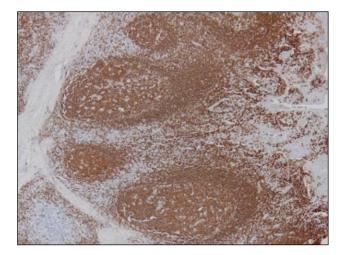
B-cell positive expression	Brown cytoplasmic staining
Kidney	Negative
Nuclei	Blue

PROCEDURE NOTES:

- 1. Do not allow sections to dry out at any point during procedure.
- 2. Dilute sufficient Hydrogen Peroxide 30%, Aqueous (Part 1206) with distilled water to a 3% (1/10) solution prior to use.
- Dilute sufficient Tris Buffered Saline 0.05M, pH 7.6, 10X (Part 140304) with distilled water to a 1/10 solution prior to use for all buffer rinses in this procedure.
- Cell Marque CD20 (L26) is the concentrated primary antibody used. Dilute primary antibody to 1/100 working dilution with Cell Marque Emerald: Antibody Diluent (936B).
- 5. Cell Marque HiDef Detection™ HRP Polymer System (954D) provides the Amplifier and HRP Polymer solutions used.
- 6. Cell Margue DAB Substrate Kit (957D) is the chromogen used.
- 7. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- 1. Cell Marque CD20 Antibody datasheet.
- 2. Cell Marque Emerald: Antibody Diluent datasheet.
- 3. Cell Marque HiDef Detection[™] Polymer System datasheet.
- 4. Cell Marque DAB Substrate Kit datasheet.
- 5. Modifications developed by Newcomer Supply Laboratory.



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