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Part 3625 Revised January 2020

p16 Control Slides – Technical Memo

CONTROL SLIDES:

Part 3625A Part 3625B 10 Slide/Set

98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining breast and negative staining adipose.

Fixation: Formalin 10%, Phosphate Buffered (Part 1090).

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: p16 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 p16 Control Slides are for the positive immunohistochemical staining of p16, a tumor suppressor protein that plays an important role in cell cycle regulation and acts as a tumor suppressor implicated in the prevention of cancers. p16 can serve as a surrogate marker for high risk HPV in cases of cervical, head and neck and a variety of HPV related carcinomas.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- Heat dry sections in oven according to your laboratory protocol. 1.
- 2. 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. See Procedure Note #1. а
- 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.
- Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 5. 6507) to reduce reagent usage and ensure tissue coverage.
- 6. Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes. See Procedure Note #2. a.
- 7. Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.
 - See Procedure Note #3. а.
- 8. Tap off excess buffer; apply p16 primary antibody. Incubate at room temperature for 30 minutes.
- Rinse slides in two changes of buffer. 9.
- Tap off excess buffer; apply Amplifier. Incubate for 10 minutes. 10.
- Rinse slides in two changes of buffer. 11.
- Tap off excess buffer; apply HRP Polymer. Incubate for 10 minutes. 12.
- Rinse slides in two changes of buffer. 13.
- Prepare required quantity of DAB substrate/chromogen. 14.
- Tap off excess buffer; apply DAB. Incubate for 5 minutes. 15.
- Rinse slides in four changes of distilled water. 16.
- Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 17. minutes.
- 18. Rinse slides in warm tap water to blue sections.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. 19. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

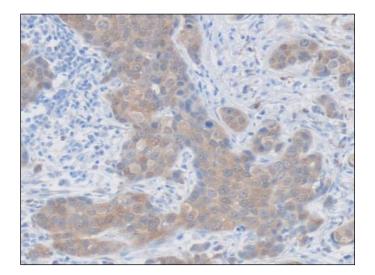
p16 positive expression	Brown nuclear & cytoplasmic staining
Adipose	Negative

PROCEDURE NOTES:

- Do not allow sections to dry out at any point during procedure. 1.
- Dilute sufficient Hydrogen Peroxide 30%, Aqueous (Part 1206) with 2. distilled water to a 3% (1/10) solution prior to use.
- Dilute sufficient Tris Buffered Saline 0.05M, pH 7.6, 10X (Part З 140304) with distilled water to a 1/10 solution prior to use for all buffer rinses in this procedure.
- 4 BD Pharmingen p16 (G175-405) is the concentrated primary antibody used. Dilute primary antibody to 1/20 working dilution with Cell Marque Emerald: Antibody Diluent (936B).
- Cell Margue HiDef Detection[™] HRP Polymer System (954D) 5. provides the Amplifier and HRP Polymer solutions used.
- 6. Cell Marque DAB Substrate Kit (957D) is the chromogen used.
- If using a xylene substitute, closely follow the manufacturer's 7. recommendations for deparaffinization and clearing steps.

REFERENCES:

- 1. BD Pharmingen p16 Antibody datasheet.
- Cell Marque Emerald: Antibody Diluent datasheet. 2.
- 3. Cell Marque HiDef Detection[™] Polymer System datasheet.
- Cell Margue DAB Substrate Kit datasheet. 4.
- 5. Modifications developed by Newcomer Supply Laboratory.



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