

## CK5/14 + p63 + P504S, Multi-Tissue Control Slides – Technical Memo

<b><u>CONTROL SLIDES:</u></b>	<b>Part 3650A</b>	<b>Part 3650B</b>
	10 Slide/Set	98 Slide/Set

### **PRODUCT SPECIFICATIONS:**

**Tissue:** P504S and CK5/14 positive staining prostate, p63 positive staining prostate and negative staining myometrium.

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

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

**Quality Control Stain:** CK5/14 + p63 + P504S quality control stained slide(s) included.

**Reactivity:** Guaranteed product specific reactivity for six months from date of receipt. Revalidate after six months 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 CK5/14 + p63 + P504S, Multi-Tissue Control Slides provide a useful combination of markers helpful in diagnosing prostatic intraepithelial neoplasia (PIN).

### **NEWCOMER SUPPLY VALIDATION PROCEDURE:**

1. Heat dry sections in oven according to your laboratory protocol.
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.
  - 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 CK5/14 + p63 + P504S multiplex primary antibody. Incubate at room temperature for 30 minutes.
9. Rinse slides in two changes of buffer.
10. Tap off excess buffer; apply MACH 2 Double Stain 2 Polymer. Incubate for 30 minutes.
11. Rinse slides in two changes of buffer.
12. Prepare required quantity of DAB substrate/chromogen.
13. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
14. Rinse slides in two changes of buffer.
15. Prepare required quantity of Vulcan Fast Red Chromogen.
16. Tap off excess buffer; apply Vulcan Fast Red. Incubate for 10 minutes.
17. Rinse slides in four changes of distilled water.
18. Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
19. Rinse slides in warm tap water to blue sections.
20. 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:**

P504S positive expression	Red granular cytoplasmic staining
CK5/14 positive expression	Brown cytoplasmic staining
p63 positive expression	Brown nuclear staining
Myometrium	Negative

### **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.
3. 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.
4. Biocare CK5/14 + p63 + P504S (PPM 225DS) is the pre-diluted multiplex primary antibody used.
5. Biocare MACH 2 Double Stain 2 Polymer Detection Kit (MRCT525) provides the polymer solution used.
6. Cell Marque DAB Substrate Kit (957D) is the chromogen used.
7. Biocare Vulcan Fast Red Chromogen Kit 2 (FR805) is the second chromogen used.
8. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### **REFERENCES:**

1. Biocare CK5/14 + p63 + P504S datasheet.
2. Biocare MACH 2 Double Stain 2 Polymer Detection Kit datasheet.
3. Cell Marque DAB Substrate Kit datasheet.
4. Biocare Vulcan Fast Red Chromogen Kit 2 datasheet
5. Modifications developed by Newcomer Supply Laboratory.

