Using Mouse-on-Mouse IHC Kits In Various Sample Types

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POLYVIEW® (MOUSE-ON-MOUSE HRP) IHC KIT (ADI-950-114)

INTRODUCTION

Accurate localization of mouse primary antibodies in mouse tissue is confounded by endogenous mouse immunoglobulins present in the tissue, which react with the anti-mouse secondary antibody reagent, causing off-target staining. In order to eliminate this background staining, reagents which block the endogenous mouse immunoglobulins and prevent non-specific staining are commonly used, and known as Mouse-on-Mouse or M.O.M. kits. As with any IHC reagent, multiple manufacturers field similar products which on their surface appear equivalent, but have widely varying performance characteristics. For this study, Enzo's Mouse-on-Mouse blocking reagent was compared to two competitive products using 3 different primary antibody IHC reagents in 3 different mouse tissues. The primary antibodies were chosen to demonstrate staining in nucleus (phospho-Akt), cytoplasm (KDEL-R), and membrane (CD31).

METHODS

IHC Assay Development

Final IHC assays for the reagents tested in this study are summarized in the table below. For each reagent, a wide range of antigen retrieval conditions, primary antibody dilutions, primary antibody incubation times, and chromogen development times were tested to arrive at conditions conducive to comparison of all 3 reagents. Mouse GI tract was used to compare M.O.M. kits with KDEL-R and p-Akt, and mouse kidney was used to compare the 3 M.O.M. reagents with CD31.

TARGET	SUPPLIER	CATALOG #	DILUTION	Ab INCUBATION (MIN.)	AR pH	AR TIME (MIN.)	CHROMOGEN INCUBATION (MIN.)
CD31	Abcam	Ab9498	1:1000	60	9	20	15 (DAB)
KDEL-R	Abcam	Ab12223	1:12000	60	9	20	10 (DAB)
pAkt	Novocastra	NCL-L-Akt-Phos	1:100	60	6	20	5 (DAB)

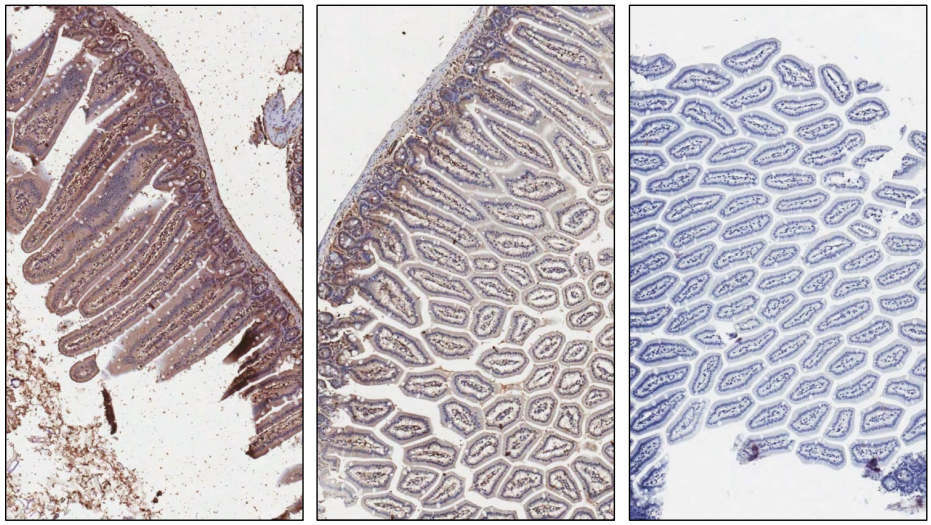
Table 1. Final IHC Assay Conditions

WHOLE SLIDE SCANNING

Whole slide scanning was performed using Aperio digital slide scanners in a third-party laboratory. All scanned images were scanned at 40x magnification and subjected to a quality control process to ensure the highest quality images possible were available for staining assessment.

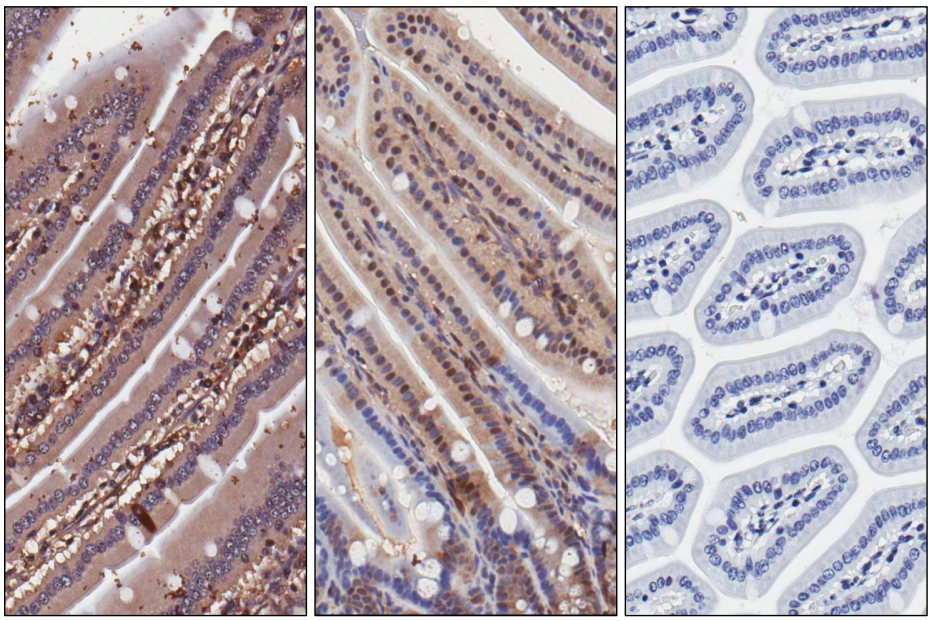
RESULTS

Side-by-side comparison of all three M.O.M. kits with each of the primary IHC reagents are shown in figures 1-6. Figure legends indicate image magnification, tissue, and antibody. In each panel, the Enzo M.O.M. reagent is shown in the center panel with two competitor's products, labeled competitor 1 and 2, in the left and right panels respectively. Staining for phospho-Akt is shown in figures 1 and 2, KDEL-R in figures 3 and 4, and CD31 in figures 5 and 6.



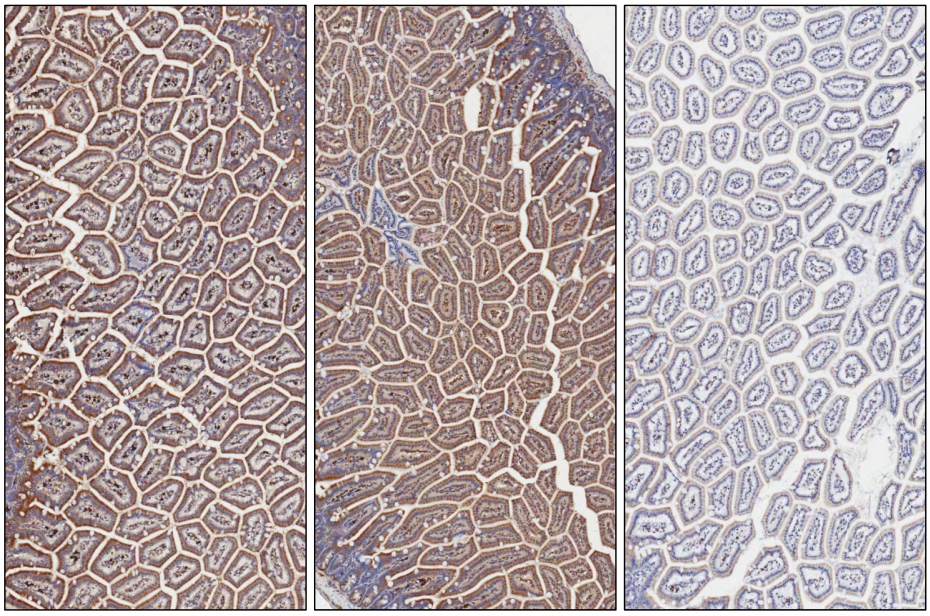
Competitor Kit 1 | Enzo M.O.M. Kit | Competitor Kit 2

Figure 1. M.O.M. Comparison Using Phospho-Akt in Mouse GI Tract – 10x magnification shown. Enzo M.O.M. Block exhibits superior performance to competitor 1 in terms of reduction of background staining, and competitor 2 in terms of maintaining the quality of the specific nuclear phospho-Akt staining.



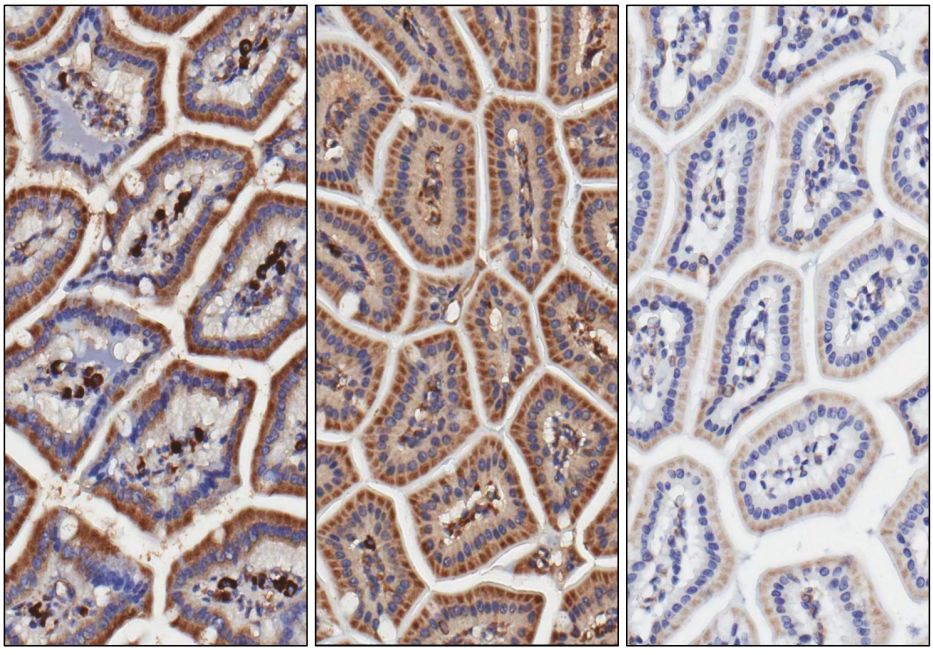
Competitor Kit 1 | Enzo M.O.M. Kit | Competitor Kit 2

Figure 2. M.O.M. Comparison Using Phospho-Akt in Mouse GI Tract – 40x magnification shown. Enzo M.O.M. Block exhibits superior performance to competitor 1 in terms of reduction of background staining, and competitor 2 in terms of maintaining the quality of the specific nuclear phospho-Akt staining.



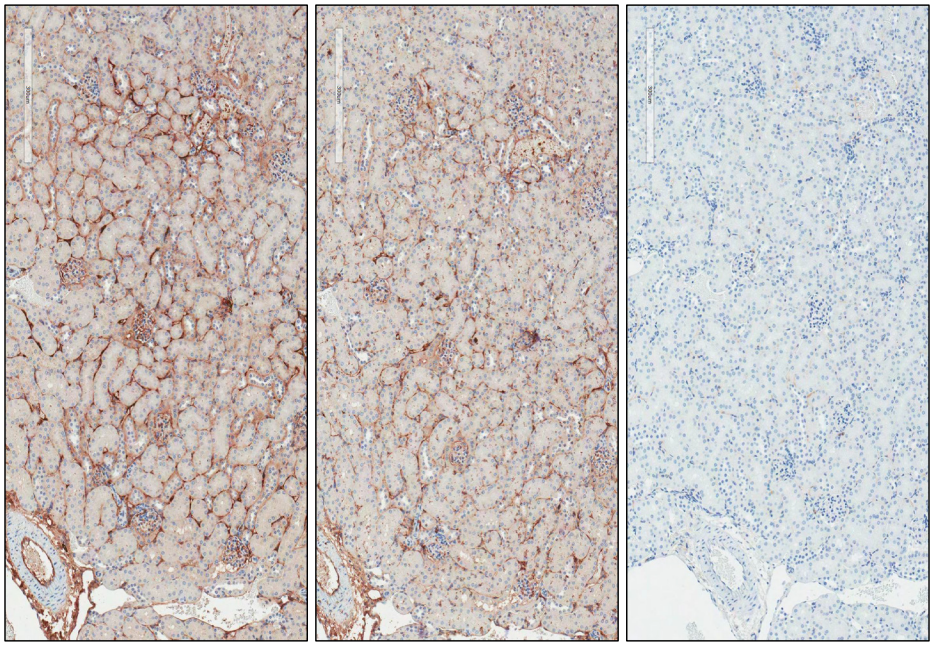
Competitor Kit 1 | Enzo M.O.M. Kit | Competitor Kit 2

Figure 3. M.O.M. Comparison Using KDEL-Receptor in Mouse GI Tract – 10x magnification shown. Enzo M.O.M. Block exhibits superior performance to competitor 1 in terms of reduction of background staining, and competitor 2 in terms of maintaining the quality of the specific cytoplasmic KDEL-Receptor staining.



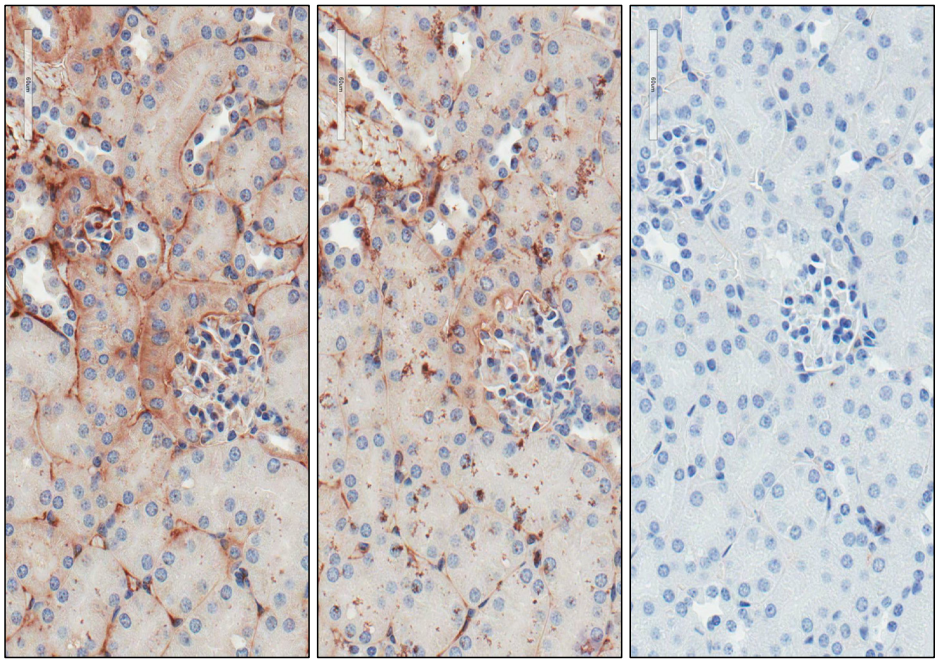
Competitor Kit 1 | Enzo M.O.M. Kit | Competitor Kit 2

Figure 4. M.O.M. Comparison Using KDEL-Receptor in Mouse GI Tract – 40x magnification shown. Enzo M.O.M. Block exhibits superior performance to competitor 1 in terms of reduction of background staining, and competitor 2 in terms of maintaining the quality of the specific cytoplasmic KDEL-Receptor staining. Note superior performance of Enzo reagent in providing clear divisions in the cytoplasm of neighboring cells.



Competitor Kit 1 | Enzo M.O.M. Kit | Competitor Kit 2

Figure 5. M.O.M. Comparison Using CD31 in Mouse Kidney – 10x magnification shown. Enzo M.O.M. Block exhibits superior performance to competitor 1 in terms of reduction of background staining, and competitor 2 in terms of maintaining the quality of the specific membranous CD31 staining.



Competitor Kit 1 | Enzo M.O.M. Kit | Competitor Kit 2

Figure 6. M.O.M. Comparison Using CD31 in Mouse Kidney – 40x magnification shown. Enzo M.O.M. Block exhibits superior performance to competitor 1 in terms of reduction of background staining, and competitor 2 in terms of maintaining the quality of the specific membranous CD31 staining. Note higher cytoplasmic background in competitor 1 image (left panel, red arrowheads) in serial sections of the same kidney, same region pictured.

DISCUSSION

The Enzo M.O.M. block kit displayed superior performance to the two competitive products tested in parallel. The Enzo product achieved the best balance of reducing background while maintaining signal integrity with three primary antibodies in two different mouse tissues. Similar patterns were observed for kit performance in all conditions, with Competitor Kit 1 consistently having higher background than the Enzo kit, and Competitor Kit 2 consistently having greatly reduced specific staining intensity. While Competitor Kit 1 and the Enzo kit appear to have roughly equivalent performance with CD31 staining in mouse kidney as the benchmark, overall the Enzo reagent was superior when considering all stains and conditions.

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