

K951965

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Summary of Safety and Effectiveness

DAKO LSAB® 2 Kit, HRP
DAKO ENVISION™ SYSTEMS, HRP

I. Introduction

Immunoenzymatic/immunohistochemical (IHC) staining techniques allow for the qualitative identification of tissue antigens. Antigens are visualized via the sequential application of a specific antibody to the antigen (primary antibody) and a detection system. Immunohistochemical detection systems usually consist of a secondary antibody to the primary antibody (link antibody), an enzyme complex and a chromogenic substrate. The enzymatic activation of the chromogen yields a visible reaction product at the antigen site. Results aid in the diagnosis of pathophysiological processes which may or may not be associated with a particular antigen.

II. Device Descriptions

The DAKO LSAB® 2 Kit, HRP (Code No. K0677) is based on a modified labeled avidin-biotin (LAB) technique in which a biotinylated secondary antibody forms a complex with peroxidase-conjugated streptavidin molecules. This kit is very similar to the original DAKO LSAB® Kit, HRP (K0680) previously cleared in submission K924726. Refinements to the LSAB® 2 Kit include the elimination of a separate protein blocking reagent incubation and reconfigured substrate-chromogen reagents.

The DAKO Envision™ Systems, HRP, contains a Peroxidase Blocking Reagent similar to the Hydrogen Peroxide provided with the LSAB® and LSAB® 2 Kits. Like the LSAB® 2 Kit, the Envision™ System does not require a protein blocking reagent incubation. The Envision™ System also eliminates the sequential applications of link antibody and streptavidin common to the LSAB® and LSAB® 2 Kits. A polymer, labeled with goat anti-mouse and goat anti-rabbit immunoglobulins conjugated to horseradish peroxidase eliminates these steps. The DAKO Envision™ System offers the user a choice of two protocols. Protocol #1 consecutively incubates the primary antibody and Peroxidase Labeled Polymer for ten (10) minutes. Protocol #2 increases the staining intensity of the Envision™ System by lengthening the incubations of the primary antibody and the labeled polymer to thirty (30) minutes. The DAKO Envision™ System, HRP, is available with DAB (Code No. K1390) or Ready-to-Use AEC substrate-chromogen (Code No. K1391). Both systems use the same Peroxidase Blocking Reagent and Peroxidase Labeled Polymer.

Concentrated primary rabbit/mouse antibodies or DAKO® Ready-to-Use N-Series Primary Antibodies and Negative Control Reagents are suitable for use with the DAKO LSAB® 2 Kit and DAKO Envision™ Systems. Primary antibodies are not included with the DAKO LSAB® 2 Kit, HRP or DAKO Envision™ Systems, and must be purchased separately by the user.

III. Experimental Data

Concurrent testing was done with the DAKO LSAB® Kit, HRP and the DAKO LSAB® 2 Kit, HRP using formalin-fixed, paraffin embedded tissues. Equivalent interpretive results were observed in all test specimens.

III. Experimental Data, continued

Similar concurrent testing was done with the DAKO LSAB[®] 2 Kit, HRP and the DAKO Envision[™] System, HRP with Ready-To-Use AEC substrate-chromogen (K1391) using formalin-fixed, paraffin embedded tissues. Equivalent interpretive results were observed in all test specimens.

This test procedure was repeated using the DAKO LSAB[®] 2 Kit, HRP and the DAKO Envision[™] System, HRP with liquid DAB substrate-chromogen (K1390). Equivalent interpretive results were observed in all test specimens.

Additional testing was done with the DAKO LSAB[®] 2 Kit, HRP and the DAKO Envision[™] System, HRP with Ready-To-Use AEC substrate-chromogen (K1391) using acetone-fixed, frozen tissues and blood smears. Results were equivalent.

Results obtained from comparative testing of the DAKO Envision[™] System, HRP with Ready-to-Use AEC substrate-chromogen using Protocols #1 and #2 support package insert claims for Protocol #2. Protocol #2 increases the staining intensity of the DAKO Envision[™] System. Concentrated Primary antibodies used with protocol #2 may be diluted up to twenty times the optimal dilution used with Protocol #1.

Comparison testing of LSAB2 with 2 component AEC versus ready-to-use AEC was performed using LCA (N1514), UCHL1 (N1520), and HMB45 (N1545). The tissues tested included tonsil (N1514, and N1520) and melanoma (N1545). Results showed that the same level of staining was obtained with both product configurations for N1545 and N1520. Slightly more intense staining (2 component AEC staining = 3-3.5 vs ready-to-use AEC staining = 4+) was noted for ready-to-use AEC with N1514. Background staining of both negative controls and positively stained tissues was evaluated as negative i.e., no staining. Thus, the LSAB2 with the ready-to-use AEC is similar or better in performance to LSAB2 with the 2-component AEC.

IV. Product Specific Limitations

DAKO LSAB[®] 2, Kit (K0677):

Endogenous avidin-binding activity (EABA) has been noted in frozen sections of liver (entire hepatic nodule) and kidney (tubular epithelium), as well as in frozen and formalin-fixed lymphoid tissues (paracortical histiocytes). EABA can be suppressed by sequential 20-minute incubations, first with 0.1% avidin and then with 0.01% biotin in 0.05 M Tris-HCl buffer, pH 7.2-7.6 prior to the application of the primary antibody, or use DAKO[®] Biotin Blocking System (code no. X0590).

DAKO Envision[™] System, HRP (K1390, K1391):

Some primary antibodies may require proteolytic digestion and target retrieval to achieve optimal staining results. Protocol #2 is recommended for use. Contact DAKO Technical Services at 800/424-0021 for additional information.

Endogenous peroxidase or pseudoperoxidase activity can be found in hemoproteins such as hemoglobin, myoglobin, cytochrome, and catalase as well as in eosinophils. This activity can be inhibited by incubating specimens with Peroxidase Blocking Reagent Bottle #1 of the DAKO Envision[™] System, HRP for five (5 ± 1) minutes prior to the application of the primary antibody. Blood and bone marrow smears and frozen tissues can also be treated with this reagent. However, this procedure does not abolish the reddish-brown pigment of hemoproteins. Alternatively, a solution of methanol-hydrogen peroxide can be used. Some antigens may become denatured with this procedure.