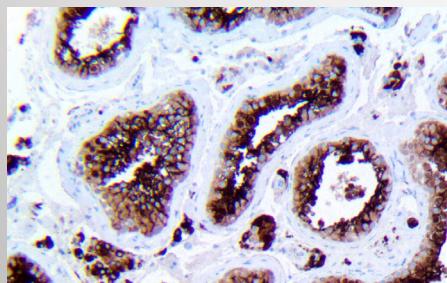


ANTIBODY PROTOCOLS FOR AUTOMATED SYSTEMS

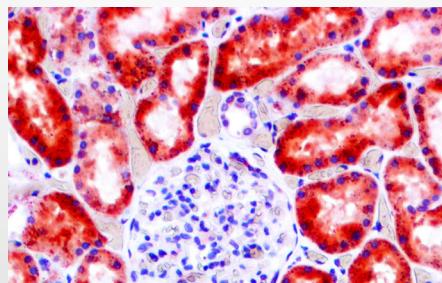


Optimized Protocols for Leica Bond Automated IHC System

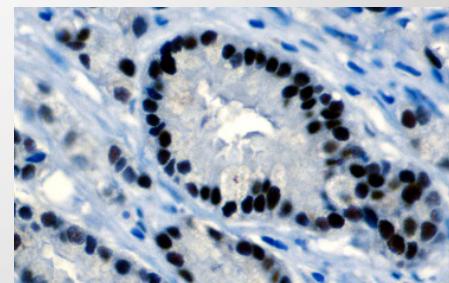
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
ACE2 (BSB-135), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ACTH (BSB-25), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Actin Muscle Specific (HHF35), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Actin Smooth Muscle (BSB-15), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Adenovirus (20/11 and 2/6), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Adipophilin/ADRP (BSB-91), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Alpha Synuclein (BSB-114), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Alpha-Fetoprotein (BSB-23), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
AMACRacemase/P504S (I3H4), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Amyloid Beta (RBT-A4), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Androgen Receptor (BSB-4), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Annexin AI (BSB-95), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Annexin VII (EP367), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
APOE4 (BSB-170), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Arginase-1 (EP261), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ARID1A (EP303), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Aspergillus (RBT-A.fumi), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ATM (EP327), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ATRX (BSB-108), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ATRX (RBT-ATRX), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
ATRX (RBT-ATRX), RMab	1:2.5	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
B7H3/CD276 (RBT-B7H3), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
B7H3/CD276 (RBT-B7H3), RMab	1:25	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min



IHC of ACE2 on FFPE SARS-CoV-2 Infected Testis Tissue



IHC of AMACRacemase on FFPE Kidney Tissue



IHC of Androgen Receptor on FFPE Prostate Tissue



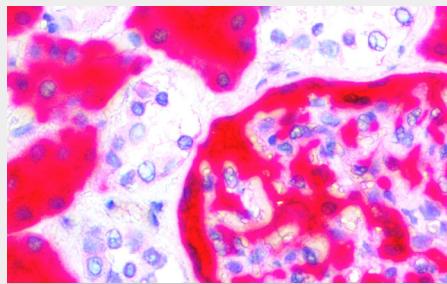
Optimized Protocols for Leica Bond Automated IHC System

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Permeabilization Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
BAPI (BSB-109), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Bax (E63), RMab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
bcl-10 (BSB-22), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
bcl-10 (BSB-22), MMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
bcl-2 (BSB-5), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
bcl-2 (EP36), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Beta-Catenin (14), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Beta-Catenin (RM276), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Beta-Catenin (RM276), RMab	1:25	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
BOB.1 (RBT BOB.1), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Brachyury (RBT-TBXT), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Brachyury (RBT-TBXT), RMab	1:100	72 °C	ER 1- Citrate	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
BRAF V600E (RM8), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
BRAF V600E (RM8), RMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
BRG-1/SMARCA4 (BSB-154), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
BRG-1/SMARCA4 (BSB-154), MMab	1:25	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
c-Met/HGFR (EP1454Y), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
c-Met/HGFR (EP1454Y), RMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
C4d (EP272), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CA-125 (OC125), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CA15-3 (DF3), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CA19-9 (121SLE), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cadherin17/Li-Cadherin (EP86), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Caldesmon (BSB-19), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Calponin (BSB-20), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Calretinin (RM324), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Candida (RBT-C.alb), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Carbonic Anhydrase 9 (EPI61), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Caveolin-1 (EP353), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD10 (56C6), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD10 (EPI95), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD117 (EP10), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD117 (RM359), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD11b (EP45), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD11c (EPI57), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD123 IL-3Ra (BSB-59), MMab	RTU	72 °C	ER 1- Citrate	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD138 (B-A38), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD138 (EP201), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD14 (7), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD14 (EPI28), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD142/TF/Coagulation Factor III (BSB-143), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

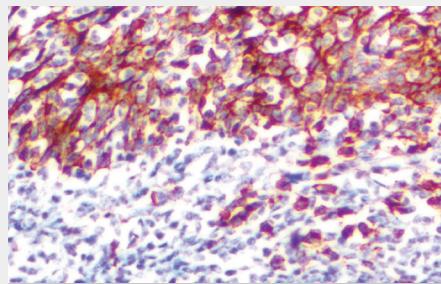
Optimized Protocols for Leica Bond Automated IHC System



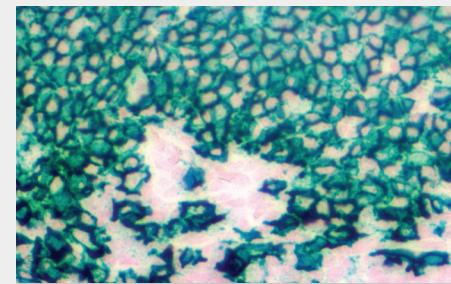
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
CD15 (BSB-119), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD15 (BSB-119), MMab	1:50	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD16 (EP364), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD163 (10D6), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD19 (BSB-97), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD19 (BSB-97), MMab	1:20	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD2 (AB75), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD20 (L26), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD21 (EP64), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD23 (IB12), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD23 (EP75), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD25 (4C9), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD25 (4C9), MMab	1:12.5	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD3 (RBT-CD3), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD30 (Ber-H2), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD30 (Ber-H2), MMab	1:75	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD31 (1A10), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min



IHC of CD10 on FFPE
Kidney Tissue



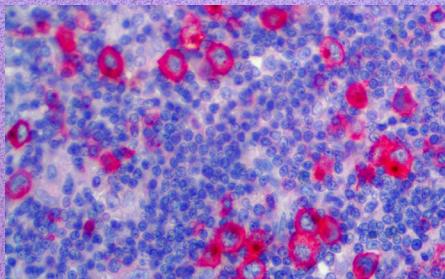
IHC of CD138 on FFPE
Tonsil Tissue



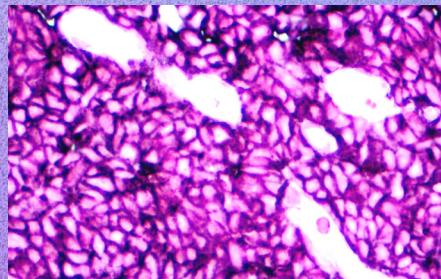
IHC of CD20 on
FFPE Colon Tissue

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
CD31 (1A10), MMab	1:50	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD33 (RBT-CD33), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD34 (EP88), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD34 (QBEnd/10), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD35 (RLB25), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD35 (RLB25), MMab	1:40	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD38 (SPC32), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD38 (SPC32), MMab	1:50	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD4 (RBT-CD4), RMab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD41/ Integrin alpha IIb (EPI78), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD42b (EP409), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

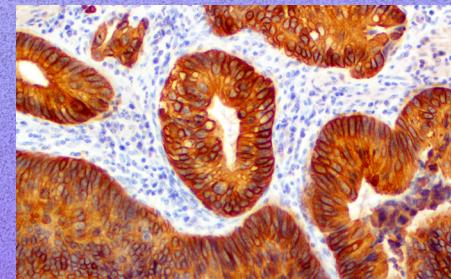
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
CD43 (MT1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD44 (BSB-12), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD45 (2B11 & PD7/26), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD45RO (UCHL-1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD5 (RBT-CD5), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD5 (RM314), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD56 (123C3.D5), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD63 (NKI/C3), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD68 (BSB-8), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD68 (KP-1), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD7 (EPI32), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD71 (JOFII), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD74 (LN2), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD75 (LN-1), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD75 (LN-1), MMab	1:100	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CD79a (JCBII7), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD8 (C8/144B), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min



IHC of CD30 on FFPE
Hodgkin's Lymphoma Tissue



IHC of CD45 on FFPE
Tonsil Tissue



IHC of Cytokeratin 20 on
FFPE Colon Adenocarcinoma Tissue

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
CD8 (EP334), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CD99 (BSB-9), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CDK2 (RBT-CDK2), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CDK2 (RBT-CDK2), RMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CDK4 (EPI80), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CDX2 (EP25), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CEA (BSB-13), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CEA (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Chromogranin A (LK2H10), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
CITED1 (BSB-177), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

Optimized Protocols for Leica Bond Automated IHC System



Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Claudin-1 (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Claudin-1 (Polyclonal), RPab	1:40	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Claudin-7 (EP399), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Clusterin (RM437), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Clusterin (RM437), RMab	1:500	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
CTLA-4/CD152 (BSB-88), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cyclin D1 (RM241), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cyclin D3 (BSB-171), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 14 (LL002), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 14 (RM328), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 14 (RM328), RMab	1:75	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 17 (BSB-33), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 18 (EP30), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 19 (BSB-34), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 19 (RM364), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 20 (EP23), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 20 (Ks20.8), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 4 (EP4), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 5 & 6 (D5 & I6B4), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 7 (OV-TL I2/30), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 8 (EPI7), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin 8 & I8 (B22.1 & B23.1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin Cocktail AE1 & AE3 (AE1 & AE3), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin HMW 34BE12 (34BetaE12), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin LMW CAM5.2 (CAM5.2), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin MNFI16 (MNFI16), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin MNFI16 (MNFI16), MMab	1:40	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytokeratin OSCAR (OSCAR), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Cytomegalovirus (8B1.2, 1G5.2 & 2D4.2), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Desmin (D33), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Desmoglein-3 (EP306), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
E-Cadherin (EP6), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
EMA (E29), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
EpCAM (Ber-EP4)	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
EpCAM/Epithelial Specific Antigen (Ber-EP4), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
EpCAM/Epithelial Specific Antigen (MOC-31), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

* HIER (Pressure Cooker at 115 °C x 10-15 min or PT Module or Water bath at 98°C x 30-45 min) prior to detecting



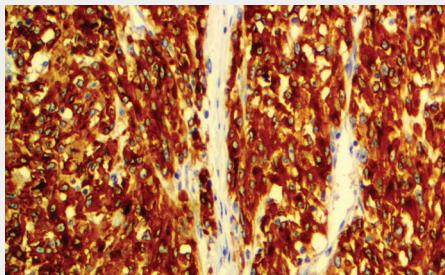
Optimized Protocols for Leica Bond Automated IHC System

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Epstein Barr Virus LMP-1 (CSI-4), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ERCC1 (EP219), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ERG (EP111), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Estrogen Receptor (BSB-1), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Estrogen Receptor (RM292), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Factor VIII-Related Antigen (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Factor XIIIa (EP292), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Fascin (BSB-36), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Fli-1 (G146-222), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
FOXA1/HNF-3A (EP277), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
FSH (BSB-55), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Fumarate Hydratase/Fumarase (BSB-151), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Fumarate Hydratase/Fumarase (BSB-151), MMab	1:12.5	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Galectin-3 (9C4), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Gastrin (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Gastrin (Polyclonal), RPab	1:300	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
GATA3 (EP368), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
GATA3 (L50-823), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
GCDFP-15 (23A3), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
GFAP (G-A-5), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Glucagon (BSB-111), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
GLUT-1 (RBT-GLUT1), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
GLUT1 (EP141), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Glutamine Synthetase (GS-6), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Glycophorin A (GA-R2 (HIR2)), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Glypican-3 (IGI2), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Glypican-3 (IGI2), MMab	1:250	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Granzyme B (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
hCG (BSB-38), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HE4 (EP370), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HEG1 (SKM9-2), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
HEG1 (SKM9-2), MMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Helicobacter Pylori (BSB-37), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Helicobacter Pylori (EP279), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Helicobacter pylori (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Hepatitis B Virus Core Antigen (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Hepatitis B Virus Surface Antigen (AI0f1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

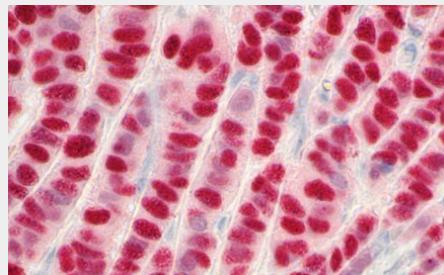
Optimized Protocols for Leica Bond Automated IHC System



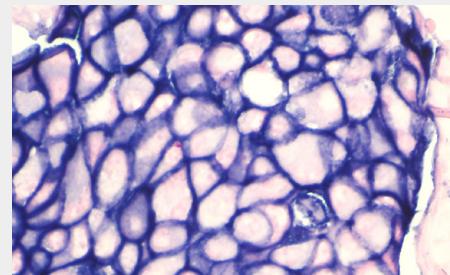
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Hepatocyte Specific Antigen/ Hep-Par1 (OCHIE5), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HER neu (EP3)	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HER-2 neu (RBT-HER2), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Herpes Simplex Virus I (10A3), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Herpes Simplex Virus I (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Herpes Simplex Virus I & II (10A3 & BSB-116), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Herpes Simplex Virus I & II (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Herpes Simplex Virus II (BSB-116), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Herpes Simplex Virus II (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HHV-8 (13B10), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HIF-1alpha (EP118), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Histone H3 Phospho (EP233), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Histone H3 Phospho (EP233), RMab	1:150	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
hPL (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min



IHC of DOG1 on FFPE Gastrointestinal Stromal Tumor Tissue



IHC of Estrogen Receptor on FFPE Breast Tissue

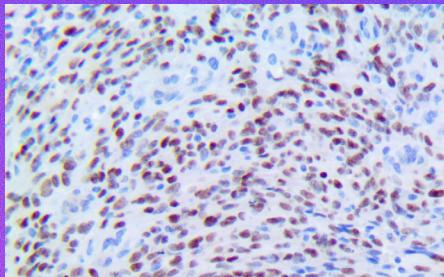


IHC of Her-2 neu on FFPE Breast Carcinoma Tissue

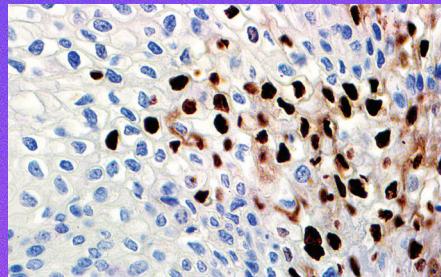
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
HPV (BSB-66), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
HPV16 (CAMVIR-1), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
ICOS/CD278 (RM417), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
ICOS/CD278 (RM417), RMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
IDH1 RI32H (IHC132), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IDH1 RI32H (RBT-IDH1), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgA (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgA (Polyclonal), RPab	1:250	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgG (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

* HIER (Pressure Cooker at 115 °C x 10-15 min or PT Module or Water bath at 98°C x 30-45 min) prior to detecting

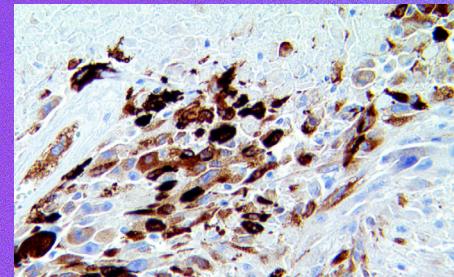
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
IgG (Polyclonal), RPab	1:100	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgG4 (BSB-96), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgG4 (EPI38), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgM (BSB-41), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgM (BSB-41), MMab	1:100	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
IgM (Polyclonal), RPab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
INI-1 (25), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
INI-1 (RBT-INII), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
INI-1 (RBT-INII), RMab	1:12.5	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
INSM1 (BSB-123), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
INSM1 (RBT-INSM1), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Insulin (BSB-42), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Kappa (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Kappa (Polyclonal), RPab	1:15	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min



IHC of HHV-8 on FFPE
Kaposi's Sarcoma Tissue



IHC of HPV on FFPE
Infected Cervix Tissue



IHC of IDH1 on
FFPE Glioblastoma Tissue

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Kappa Light Chains (BSB-58), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Ki-67 (EP5), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Ki-67 (RM360), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Lambda (BSB-16), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Lambda (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Lambda (Polyclonal), RPab	1:250	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Laminin-R/RPSA (BSB-144), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Laminin-R/RPSA (BSB-144), MMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Langerin (12D6), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
LEF1 (EP310), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
LEF1 (RBT-LEF1)	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
LEF1 (RBT-LEF1)	1:100	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min

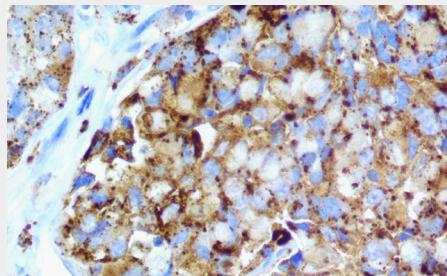
Optimized Protocols for Leica Bond Automated IHC System



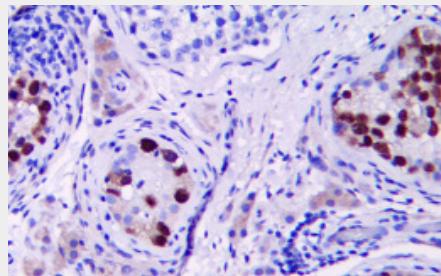
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
LH (BSB-53), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
LIN28 (EP150), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
LIN28 (EP150), RMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
LMO2 (RBT-LM02), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
LMO2 (RBT-LM02), RMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Lysozyme (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MAGEC2 (EP405), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Mammaglobin (EP249), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Mart-1/Melan-A (A103), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Mart-1/Melan-A (M2-7C10), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Maspin (BSB-92), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Melanoma Cocktail: HMB-45, MART-1 & Tyrosinase (HMB-45, A103 & BSB-6), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Melanoma KBA.62 (KBA.62), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Melanoma KBA.62 (KBA.62), MMab	1:75	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Melanosome HMB45 (HMB-45), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Mesothelial Cell (HBME-1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MGMT/AGAT (EP337), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MGMT/AGAT (EP337), RMab	1:15	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MiTF (C5 & D5), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MiTF (C5 & D5), MMab	1:100	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MLHI (G168-728), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MSH2 (BSB-147), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MSH2 (RBT-MSH2), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MSH6 (44), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MSH6 (EP49), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MTAP (RBT-MTAP), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MTAP (RBT-MTAP), RMab	1:25	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MUC1 (BSB-44), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC1 (EP85), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC2 (BSB-45), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC2 (BSB-45), MMab	1:400	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC4 (8G7), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC4 (8G7), MMab	1:25	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC5AC (CLH2), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUC6 (CLH5), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MUMI (EPI90), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Musashi 2 (RM422), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

* HIER (Pressure Cooker at 115 °C x 10-15 min or PT Module or Water bath at 98°C x 30-45 min) prior to detecting

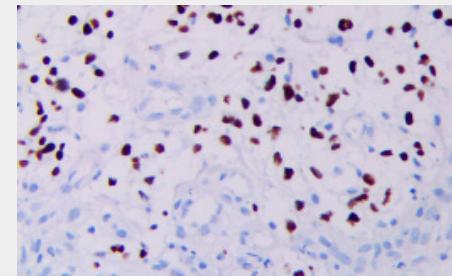
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Mycobacterium tuberculosis (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myelin Basic Protein (EP207), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myeloperoxidase (EPI51), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myeloperoxidase (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myeloperoxidase/MPO (BSB-180), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
MyoD1 (EP212), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MyoD1 (EP212), RMab	1:100	72 °C	ER 2- EDTA	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MYOD1 (RBT-MYOD1)	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
MYOD1 (RBT-MYOD1)	1:150	72 °C	ER 2- EDTA	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Myogenin (F5D), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myoglobin (EP87), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myosin Smooth Muscle (BSB-17), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Myosin Smooth Muscle (BSB-17), MMab	1:400	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Napsin A (BSB-II2), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Napsin A (EP205), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Nestin (EP287), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min



IHC of Napsin A on FFPE Lung Adenocarcinoma Tissue



IHC of OCT-4 on FFPE Testis Tissue



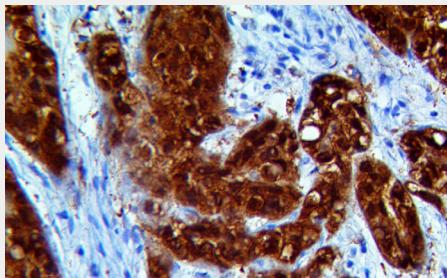
IHC of OLIG2 on FFPE Neuroblastoma Tissue

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
NeuN (A60), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
NeuN (RBT-NeuN), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Neurofilament (2FII), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Neurofilament (2FII), MMab	1:250	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
NGFR (BSB-18), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
NKX2.2 (EP336), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
NKX2.2 (EP336), RMab	1:33	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
NKX3.1 (EP356), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
NKX3.1 (RM430), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

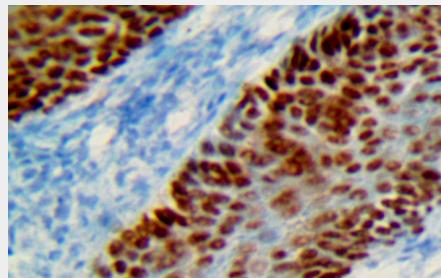
Optimized Protocols for Leica Bond Automated IHC System



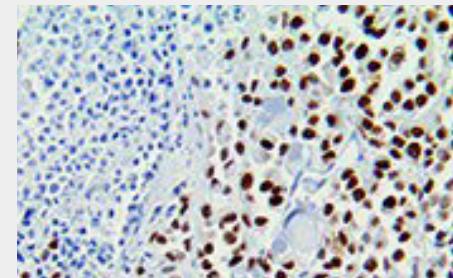
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
NRAS (RBT-NRAS), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
NSE (BSB-94), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
OCT-2 (EPI15), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
OCT-2 (EPI15), RMab	1:75	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
OCT-4 (RBT-OCT4), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
OLIG2 (EPI12), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
OLIG2 (RBT-OLIG2), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Osteonectin/SPARC (BSB-93), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
OX-40/CD134 (BSB-90), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min



IHC of p16 on FFPE
Transition Cell Carcinoma Tissue



IHC of p63 on FFPE
Basal Cell Carcinoma Tissue

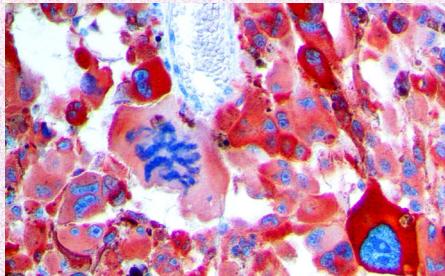


IHC of PRAME on FFPE
Melanoma Tissue

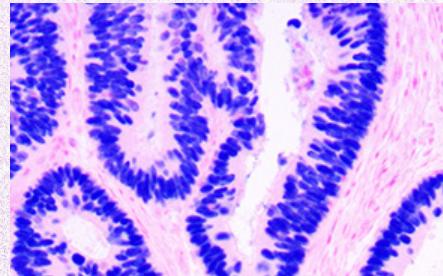
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
OX-40/CD134 (BSB-90), MMab	1:10	72 °C	ER 2- EDTA	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
p16 (16P04, JC2)	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p16 (RBT-p16), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p16 (RM267), RMab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
p21 (DCS-60.2), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p21 (DCS-60.2), MMab	1:12.5	72 °C	ER 2- EDTA	100°C x 30 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p27 (SX53G8), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
p27 (SX53G8), MMab	1:10	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
p40 (ZR8), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p53 (DO7), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p57 (Kp10), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p63 (4A4), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p63 (4A4), MMab	1:75	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
p63 (EPI74), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Pan-trk (RM423), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

* HIER (Pressure Cooker at 115 °C x 10-15 min or PT Module or Water bath at 98°C x 30-45 min) prior to detecting

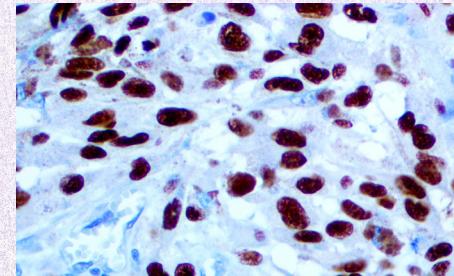
Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Parafibromin (BSB-50), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Parathyroid Hormone/PTH (BSB-24), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Parvovirus (R92F6), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PAX-5 (RBT-PAX5), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PAX-5 (RBT-PAX5), RMab	1:50	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PAX-8 (RM436), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PAX-8 (RM436), RMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PAX-8 (ZR-1), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PD-1/CD279 (NAT-105), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PD-L1/CD274 (28-8), RMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PD-L1/CD274 (RBT-PDL1), RMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PDGFR-B (RBT-PDGFRB), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PDGFR-B (RBT-PDGFRB), RMab	1:10	72 °C	ER 2- EDTA	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Perforin (5B10), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min



IHC of S100 on FFPE Melanoma Tissue



IHC of SATB2 on FFPE Colon Carcinoma Tissue



IHC of SOX-10 on FFPE Melanoma Tissue

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
PGP 9.5 (BSB-46), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PHOX2B (EP312), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PLA2RI (BSB-129), MMab	RTU	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PLAP (BSB-47), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PLAP (EPI94), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PMS2 (EP51), RMab	RTU	72 °C	ER 1- Citrate	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PMS2 (RBT-PMS2)	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Podoplanin/D2-40 (D2-40), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PRAME (RBT-PRAME), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Prealbumin/Transthyretin (BSB-125), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Progesterone Receptor (BSB-2), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min

Optimized Protocols for Leica Bond Automated IHC System



Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
Progesterone Receptor (BSB-2), MMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Progesterone Receptor (RBT-22)	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Prolactin (EPI93), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Prolactin (PRL02), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PSA (BSB-7), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PSA (RBT-PSA), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PSAP (PASE/4LJ), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
PTEN (RM265), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
PU.1 (EPI18), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Renal Cell Carcinoma (PN-15), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
S-100 (4C4.9), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
S100A1 (EPI84), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
S100P (EPI86), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
S100P (RBT-S100P), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SALL4 (6E3), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SALL4 (6E3), MMab	1:50	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SARS-CoV-2 (BSB-134), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SATB2 (EP281), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SDHB (BSB-131), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
SDHB (BSB-131), MMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
SF-1/Steroidogenic Factor 1 (BSB-149), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SF-1/Steroidogenic Factor 1 (BSB-149), MMab	1:50	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SMAD4/DPC4 (RBT-SMAD4), RMab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
SOX-10 (EP268), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SOX-9 (EP317), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
SS18-SSX (RBT-SS18-SSX), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
STAT6 (RM473), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Stathmin (EP247), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Stathmin (EP247), RMab	1:25	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
SV40 (Pab101), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Synaptophysin (EPI58), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Synaptophysin (Polyclonal), RPab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Synaptophysin (Polyclonal), RPab	1:75	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Tau (BSB-115), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TCL1 (EPI05), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TCR Alpha (BSB-126), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TCR Beta (BSB-117), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

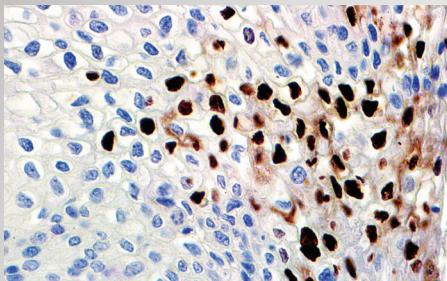
* HIER (Pressure Cooker at 115 °C x 10-15 min or PT Module or Water bath at 98°C x 30-45 min) prior to detecting



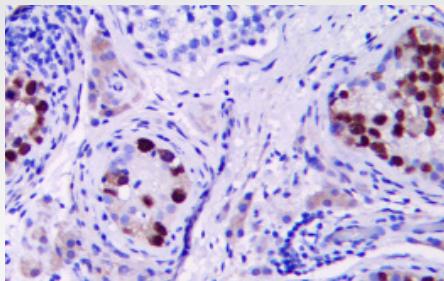
Optimized Protocols for Leica Bond Automated IHC System

Antibody Name	Dilution	Dewax Temperature	Type of Retrieval	Retrieval Time & Temperature	Peroxidase Block Time at Room Temperature	Antibody Time at Room Temperature	Post Antibody Time at Room Temperature	Polymer, Time at Room Temperature	Chromogen Time at Room Temperature	Hematoxylin Time at Room Temperature
TDP-43 / TARDBP (BSB-166), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TDP-43 / TARDBP (BSB-166), MMab	1:50	72 °C	ER 1- Citrate	100°C x 45 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TdT (EP266), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TdT (EP266), RMab	1:33	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TFE3 (EP285), RMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TFE3 (EP285), RMab	1:15	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TF3 (BSB-181), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Thyroglobulin (2H11 & 6E1), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Thyroid Peroxidase (EPI59), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TIA-1 (RBT-TIA1), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TIA-1 (TIA-1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TLE1 (1F5), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TLE1 (1F5), MMab	1:12.5	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TLE1 (BSB-142), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TLE1 (BSB-142), MMab	1:15	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TLE1 (RBT-TLE1), RMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TMPRSS2 (BSB-136), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TMPRSS2 (BSB-136), MMab	1:15	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
TORC3 (BSB-175), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Toxoplasma gondii (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TRACp (9C5), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TRACp (9C5), MMab	1:150	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Treponema pallidum (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TRPS1 (EP392), RMab	RTU	72 °C	ER 1- Citrate	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Tryptase (G3), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TSH (BSB-56), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
TTF-1 (8G7G3/1), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
Tyrosinase (BSB-6), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Uroplakin III (Polyclonal), RPab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Varicella Zoster Virus (SG1-1), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
VEGFA (RBT-VEGFA), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Villin (CWWB1), MMab	RTU	72 °C	ER 2- EDTA	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Vimentin (EP21), RMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
Vimentin (V9), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
WT1 (6F-H2), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
WT1 (6F-H2), MMab	1:175	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min
YAPI (BSB-146), MMab	RTU	*HIER	ER 1- Citrate	*HIER	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
YAPI (BSB-146), MMab	1:10	72 °C	ER 2- EDTA	100°C x 30 min	5 min	30 min	8 min	8 min	DAB, 10 min	5 min
ZFAND3 (BSB-169), MMab	RTU	72 °C	ER 1- Citrate	100°C x 20 min	5 min	15 min	8 min	8 min	DAB, 10 min	5 min

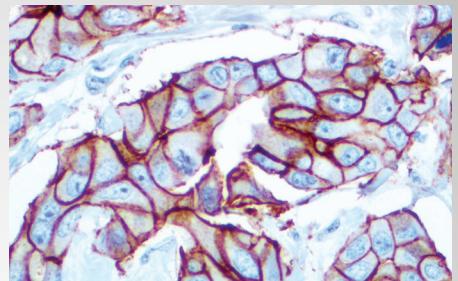
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IHC of HPV on FFPE
Infected Cervix Tissue



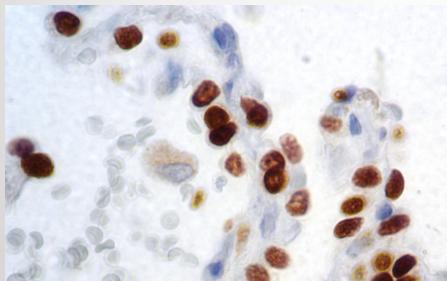
IHC of OCT-4 on FFPE
Testis Tissue



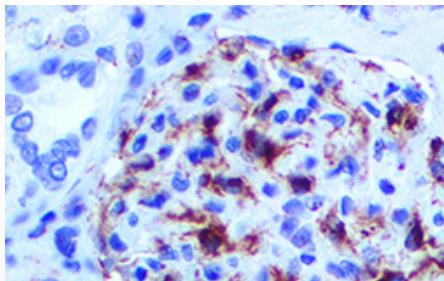
IHC of Her-2 neu on FFPE
Breast Carcinoma Tissue

Bio SB performs R&D, production, distribution and marketing of unique products for IHC, ICC, FISH and CISH that meet the highest international standards for applications in Molecular Pathology, Cancer Diagnostics & Research, Microbiology, Immunology and Genetics.

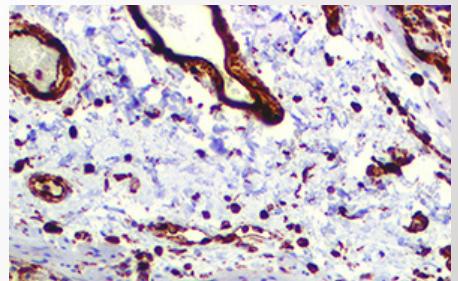
BIO SB manufactures and develops products in accordance with FDA QSR 21 CFR Part 820 cGMP, IVDR and ISO 13485:2016 standards. These guidelines enable us to produce an IVD product that meets the highest in vitro diagnostic standards.



IHC of TTF-1 on FFPE
Lung Tissue



IHC of VEGF on FFPE
Kidney Tissue



IHC of Vimentin on FFPE
Colon Tissue

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