

Assay Information

Antibody

Target ID	Target	Vendor	Cat #	Species	Clone
N/A	PB-223	BosterBio	PB-223	Human	AHF18095
N/A	NEO-102	BosterBio	NEO-102	Human	N/A

Assay Conditions

Platform	Target	Sample	Antibody	Epitope Retrieval	Primary Ab Incubation	Ab Dilution	Detection	Protocol ID*
Leica Bond Rx	PB-223	BCN601a-1	PB-223	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	PB-223	BCN721b-1	PB-223	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	PB-223	BR1102-1	PB-223	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	PB-223	nHMT25-01A	PB-223	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	PB-223	OV90 & cHMT25-01A	PB-223	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)

*See next page for protocol details

Assay Information

Assay Conditions

Platform	Target	Sample	Antibody	Epitope Retrieval	Primary Ab Incubation	Ab Dilution	Detection	Protocol ID*
Leica Bond Rx	NEO-102	BCN601a-1	NEO-102	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	NEO-102	BCN721b-1	NEO-102	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	NEO-102	BR1102-1	NEO-102	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	NEO-102	nHMT25-01A	NEO-102	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	NEO-102	OV90 & cHMT25-01A	NEO-102	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	Negative Control	BCN601a-1	Secondary Only	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	Negative Control	BCN721b-1	Secondary Only	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	Negative Control	BR1102-1	Secondary Only	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	Negative Control	nHMT25-01A	Secondary Only	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)
Leica Bond Rx	Negative Control	OV90 & cHMT25-01A	Secondary Only	DIVA Soln for 20m (110C)	1hr Ab Incubation (RT)	1 ug/mL	Biocare Human-on-Human HRP Detection System	Manual IHC (Biocare Hs-on-Hs-A01)

*See next page for protocol details

Protocol Details

Protocol ID: Manual IHC (Biocare Hs-on-Hs-A01)

Pre-processing (offline)

Step	Temperature	Time
Baking	60°C	30 min
Xylene	Ambient	5 min
Xylene	Ambient	5 min
Xylene	Ambient	5 min
Xylene	Ambient	5 min
100% Ethanol	Ambient	30 sec
100% Ethanol	Ambient	30 sec
Air dry	Ambient	Until dry

Epitope Retrieval (offline)

Step	Temperature	Time
DIVA Decloaker Solution (1X, RTU)	Ambient	0
DIVA Decloaker Solution (1X, RTU)	110°C	Varies
dH2O	Ambient	0
dH2O	0°C	20 min
TBS/T	Ambient	0
TBS/T	Ambient	0
Peroxide Block 3%	Ambient	10 min
TBS/T	Ambient	0
TBS/T	Ambient	0
TBS/T	Ambient	7 min
TBS/T	Ambient	7 min

Staining (offline)

Step	Temperature	Time
Serum Block, RTU (Invitrogen cat# 37527)	Ambient	30 min
TBS/T	Ambient	0
TBS/T	Ambient	0
TBS/T	Ambient	7 min
TBS/T	Ambient	7 min
Primary Antibody (DIG Conj. + Hs Abs)	Ambient	60 min
TBS/T	Ambient	0
TBS/T	Ambient	0
TBS/T	Ambient	7 min
TBS/T	Ambient	7 min
Mouse anti-Digoxigenin Secondary (BRR4055G)	Ambient	30 min
TBS/T	Ambient	0
TBS/T	Ambient	0
TBS/T	Ambient	7 min
TBS/T	Ambient	7 min
MACH 2 Mouse HRP-Polymer (MHRP520G)	Ambient	30 min
TBS/T	Ambient	0
TBS/T	Ambient	0
TBS/T	Ambient	7 min
TBS/T	Ambient	7 min
dIH2O	Ambient	0
DAB (CST 1:50)	Ambient	0
DAB (CST 1:50)	Ambient	5 min
dIH ₂ O	Ambient	0
dIH ₂ O	Ambient	0
dIH ₂ O	Ambient	0
dIH ₂ O	Ambient	0
dIH ₂ O	Ambient	0

Post-processing (offline)

Step	Temperature	Time
Hematoxylin (Gill 1)	Ambient	15 sec
Tap H2O rinse	Ambient	0
Bluing (0.5% NaHCO3)	Ambient	30 sec
Tap H2O rinse	Ambient	0
dH2O rinse	Ambient	0
dH2O rinse	Ambient	0
70% Ethanol	Ambient	30 sec
100% Ethanol	Ambient	30 sec
100% Ethanol	Ambient	30 sec
100% Ethanol	Ambient	30 sec
Xylene	Ambient	30 sec
Xylene	Ambient	30 sec
Xylene	Ambient	30 sec
Cytoseal XYL mount		