

Leica BOND RX™ Protocol - Anti-MASH1/Achaete-scute homolog 1 antibody [EPR19840] (ab211327)

This antibody has been successfully validated for IHC on the Leica BOND RX™ instrument using the protocol and reagents listed below. Users may further optimize the assay conditions based on their own laboratory conditions. The antibody is also suitable for manual IHC using the users' own protocol, with any suitable protocol optimisation that might be required.

Note: Protocol uses a secondary antibody not supplied as part of the Leica BOND™ reagents.

Step Name	Reagents	Temperature/Time
1 Dewax and Hydration ★	BOND™ Dewax Solution (AR9222) ◊	72°C, 0.5 min
	BOND™ Dewax Solution (AR9222) ◊	72°C, 0:00 min
	BOND™ Dewax Solution (AR9222) ◊	0:00 min
	100% Ethanol	0:00 min x 3
WASH ★	BOND™ Wash Solution (AR9590) ◊	0:00 min x 3
2 Antigen Retrieval ★	Bond™ Epitope Retrieval Solution 2 - pH 9.0 (AR9640) ◊	0:00 min x 2
	Bond™ Epitope Retrieval Solution 2 - pH 9.0 (AR9640) ◊	100°C, 20 min
WASH ★	BOND™ Wash Solution (AR9590) ◊	0:00 min x 4
	BOND™ Wash Solution (AR9590) ◊	RT, 3 min
3 Peroxide Block ★	3% (v/v) Hydrogen peroxide	RT, 10 min
WASH ★	BOND™ Wash Solution (AR9590) ◊	0:00 min x 3
4 Primary Antibody ★	ab211327 diluted in Antibody Diluent (AR9352) ◊ at 1/100 for a final concentration of 5.1 µg/mL	RT, 30 min
WASH ★	BOND™ Wash Solution (AR9590) ◊	RT, 2 min x 3
5 Secondary Antibody-1 ★	Rabbit specific IHC polymer detection kit HRP/DAB-Amplifier (ab209101)	RT, 10 min
WASH ★	BOND™ Wash Solution (AR9590) ◊	RT, 1 min x 3
	BOND™ Wash Solution (AR9590) ◊	0:00 min
6 Secondary Antibody-2 ★	Rabbit specific IHC polymer detection kit HRP/DAB-Amplifier (ab209101)	RT, 10 min
WASH ★	BOND™ Wash Solution (AR9590) ◊	RT, 1 min x 3
	Deionized Water	0:00 min
7 Chromogenic Reaction ★	DAB Kit	RT, 1:00 min
WASH ★	Deionized Water	0:00 min x 3
8 Counterstain ★	Hematoxylin	RT, 7 s (adjust as needed)
WASH ★	Deionized Water	0:00 min
	BOND™ Wash Solution (AR9590) ◊	0:00 min
	Deionized Water	0:00 min
9 Dehydration ☆	75% Ethanol	RT, 3 min
	95% Ethanol	RT, 3 min
	100% Ethanol	RT, 3 min x 2
	Xylene (Histological clearing agent can be used as an alternative)	RT, 5 min x 2
10 Mounting ☆	Commercial neutral mounting medium	

◊ Reagents provided by Leica Biosystems but not included in BOND™ Polymer Refine Detection Kit

★ Steps performed on BOND RX™ Autostainer

☆ Steps performed offline

RT - Room temperature

0:00 min - Rinse step at room temperature

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