

BOND RX General Protocol

Step	Reagents	Method
Dewax	BOND Dewax Solution (AR9222), alcohol, BOND Wash solution (AR9590)	Dewax
Antigen retrieval	BOND Epitope Retrieval Solution ER2 (HIER 2, pH 9.0) (AR9640)	20 min, 100°C
Peroxide block	3-4% (v/v) Hydrogen peroxide	5 min
Wash	BOND Wash Solution	0 min (3x)
Primary antibody	Primary antibody diluted in Bond Primary Antibody Diluent (#AR9352) to final concentration	15 min
Wash	BOND Wash Solution	0 min (4x)
Secondary antibody	BOND Polymer Refine Detection (DS9800)	8 min
Wash	BOND Wash Solution	4 min (2x)
	Deionized Water	0 min (1x)
Visualization	Mixed DAB Refine (DS9800)	0 min (1X)
	Mixed DAB Refine (DS9800)	10 min
Wash	Deionized Water	0 min (3x)
Counterstain	Hematoxylin (DS9800)	5 min
Wash	Deionized Water	0 min (1x)
	BOND Wash Solution	0 min (1x)
	Deionized Water	0 min (1x)

Table 1. IHC staining protocol on BOND RX Research Stainer (Leica Biosystems). The protocol used is the same as the default IHC protocol F on BOND RX Research Stainer (Leica Biosystems), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature. Number of washes included in the brackets.

For technical support contact information, visit: www.abcam.com/contactus