## **Enzyme Substrate Combinations**

## **Recommended Order of Substrates in Double Labeling Protocol**

This enzyme substrate combination chart is designed as a reference for optimal multiple labeling, because the order of the two colored precipitates can significantly affect the quality, color, and labeling pattern of each antigen in the stained section. This chart ensures that distinct colors are visible after the labeling reactions are completed using an optimized multiple labeling protocol. See tissue section staining examples using the combinations indicated throughout this brochure.

Second Substrate		Alkaline Phosphatase			Peroxidase					
F	irst Substrate	ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100	Vector Blue (blue) SK-5300	BCIP/NBT (indigo) SK-5400	ImmPACT VIP & Vector VIP (purple) SK-4605 & SK-4600	ImmPACT DAB, ImmPACT DAB EqV & DAB (brown) SK-4105, SK-4103, SK-4100	DAB-Ni (gray-black) SK-4100	ImmPACT NovaRED & Vector NovaRED (red) SK-4805 & SK-4800	ImmPACT SG & SG (blue-gray) SK-4705 & SK-4700	ImmPACT AEC, ImmPACT AMEC Red & AEC (red) SK-4205, SK-4285, SK-4200
Alkaline Phosphatase	ImmPACT Vector Red & Vector Red (magenta) SK-5105 & SK-5100					+	+		+	
	Vector Blue (blue) SK-5300	+			+	+	+	+	+	+
	BCIP/NBT (indigo) SK-5400	+			+	+	+	+	+	+
Peroxidase	ImmPACT VIP & Vector VIP (purple) SK-4605, SK-4600		+			+	+		+	
	ImmPACT DAB, ImmPACT DAB EqV & DAB (brown) SK-4105, SK-4103, SK-4100	+	+	+	+				+	+
	DAB-Ni (gray-black) SK-4100	+			+	+		+		
	ImmPACT NovaRED & Vector NovaRED (red) SK-4805, SK-4800		+	+		+	+		+	
	ImmPACT SG & SG (blue-gray) SK-4705, SK-4700	+			+	+				+
	ImmPACT AEC, ImmPACT AMEC Red & AEC (red) SK-4205, SK-4285, SK-4200					+			+	

+ Indicates good contrast

Indicates incompatibility of substrates for various reasons

## For more information please visit

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