

# PROTOCOL CALENDAR

● Hands-on Time 
 ● Shift Time 
 ● Incubation Time

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	<b>DAY 1:</b> Dehydration 1 <span style="color: #f08080;">●</span> 3.5 hrs <span style="color: #ff4500;">●</span> 5 hrs <span style="color: #4682b4;">●</span> Overnight .....	<b>DAY 2:</b> Bleaching <span style="color: #f08080;">●</span> 1.25 hrs <span style="color: #ff4500;">●</span> 2 hrs <span style="color: #4682b4;">●</span> Overnight .....	<b>DAYS 3-4:</b> Rehydration, Cutting, and Permeabilization <span style="color: #f08080;">●</span> 2.5 hrs <span style="color: #ff4500;">●</span> 5.5 hrs <span style="color: #4682b4;">●</span> 48 hrs (2 days) .....		<b>DAYS 5-8:</b> Blocking <span style="color: #f08080;">●</span> 1 hr <span style="color: #ff4500;">●</span> 1.5 hrs <span style="color: #4682b4;">●</span> 96 hrs (4 days) .....	
Blocking (continued)		<b>DAYS 9-15:</b> Primary Antibody Incubation <span style="color: #f08080;">●</span> 2 hrs <span style="color: #ff4500;">●</span> 2 hrs <span style="color: #4682b4;">●</span> 168 hrs (7 days) .....				
Primary Antibody Incubation (continued)		<b>DAY 16:</b> Wash Out of Primary <span style="color: #f08080;">●</span> 2 hrs <span style="color: #ff4500;">●</span> 8 hrs <span style="color: #4682b4;">●</span> Overnight .....	<b>DAYS 17-23:</b> Secondary Antibody Incubation <span style="color: #f08080;">●</span> 2 hrs <span style="color: #ff4500;">●</span> 4.5 hrs <span style="color: #4682b4;">●</span> 168 hrs (7 days) .....			
Secondary Antibody Incubation (continued)			<b>DAY 24:</b> Wash Out of Secondary <span style="color: #f08080;">●</span> 2 hrs <span style="color: #ff4500;">●</span> 8 hrs <span style="color: #4682b4;">●</span> Overnight .....	<b>DAY 25:</b> Dehydration 2 <span style="color: #f08080;">●</span> 3 hrs <span style="color: #ff4500;">●</span> 9 hrs <span style="color: #4682b4;">●</span> Overnight .....	<b>DAY 26:</b> Final Clearing Step <span style="color: #f08080;">●</span> 2 hrs <span style="color: #ff4500;">●</span> 5 hrs Done. Store in dark.	