

RNAScope Experiment Checklist for FRESH FROZEN Samples	
Method: Multiplex V2	
Experiment Name	Sample Info
DATE:	

Sample Preparation Steps

1	Post-fixation	4% PFA, cold	15min	4°C
2	Rinse X2	PBS	Briefly	RT
3	Dehydration			
		a 50% EtOH	5min	RT
		b 70% EtOH	5min	RT
		c 100% EtOH	5min	RT
		d 100% EtOH	5min	RT
4	Drying	NA	5min	RT
5	Create Barriers	NA	5min	RT
6	Peroxidase Inactivation	H2O2 (kit)	10min	RT+hum
7	Rinse	DW	Briefly	RT
8	Digestion	Protease IV (kit)	30min	RT+hum
9	Rinse X2	PBS	Briefly	RT

Coplin Jars

V2 Protocol

Prepare 3 L 1 X Wash Buffer

Warm up RNAScope probe stocks and diluent at 40 °C for about 10 min

Prepare target probe with warmed probe stocks and diluent, mix well and cool to RT before use

Set HybEZOven and prepared humidity control tray at 40°C

Set RNAScope Amp 1-3 and HRP-C1,2,3 reagents at RT

10	Probe Incubation		2 hrs	40°C
11	Wash X2	Wash Buffer	2min	RT
12	Amplification	(kit reagents)		
		a) AMP1	30min	40°C
		b) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		c) AMP2	30min	40°C
		d) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		e) AMP3	15min	40°C
		f) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		g) HRP-C1	15min	40°C
		h) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		i) Opal	30min	40°C
		j) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		k) HRP Blocker	15min	40°C
		l) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		m) HRP-C2	15min	40°C
		n) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		o) Opal	30min	40°C
		p) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		q) HRP Blocker	15min	40°C
		r) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		s) HRP-C3	15min	40°C
		t) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		u) Opal	30min	40°C
		v) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
		w) HRP Blocker	15min	40°C
		x) <i>WASH Buffer</i>	<i>2x2min</i>	<i>RT</i>
13	Nuclear Staining	DAPI	30s	RT
14	Cover Glass	Prolong Gold		