

RNAScope Experiment Checklist for FIXED FROZEN Samples					
Method Multiplex V2					
Experiment Name			Sample Info		
DATE:					
Sample Preparation Steps					
1	OCT Removal	1X PBS	5min	RT	In Coplin Jars
2	Drying	NA	30min	60°C	
3	Post-fixation	4% PFA, cold	15min	4°C	
4	Dehydration				
		a 50% EtOH	5min	RT	In Staining Rack
		b 70% EtOH	5min	RT	
		c 100% EtOH	5min	RT	
		d 100% EtOH	5min	RT	
5	Drying	NA	5min	RT	
6	Peroxidase Inactivation	H2O2 (kit)	10min	RT	
7	Rinse	DW	Briefly	RT	
8	Acclimate to 99°C	DW preheated in steamer	Briefly	99°C	
9	Target Retrieval	Retrieval Buffer (kit)	5min	99°C	
10	Rinse	DW	Briefly	RT	
11	Dehydration	100% EtOH	3min	RT	
12	Drying	NA	5min	60°C	
13	Create Barriers	NA	5min to O/N	RT	
14	Digestion	Protease IV (kit)	30min	40°C	
15	Rinse X2	DW	Briefly	RT	

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

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