

Ordering Checklist for RNAscope™ Multiomic LS Assay for Leica Biosystems BOND RX

Assay Design Overview

Use this table to plan the channels needed for your assay based on your desired targets. Note that antibodies are available in specific channels. Select individual RNAscope primary antibodies, which do not require secondaries, from available pre-configured panels. Probes can be designed to any open channel.

Note: this checklist does not include materials needed for the preparation of the tissue blocks (sample fixation, embedding, sectioning). Tissue should be sectioned on Superfrost Plus slides. For complete instructions, see the user manual. For questions or support, contact your ACD representative at +1 (877) 576-3636.

Target	RNAscope channel					
	C1	C2	C3	C4	C5	C6
Protein targets with your primaries and RNAscope secondaries.	User rabbit primary + RNAscope anti-rabbit-secondary	Your mouse primary + RNAscope anti-mouse-secondary	-	-	-	ProximityScope antibodies
Protein targets selected from RNAscope primary antibodies. No secondaries necessary.	-	-	RNAscope primary C3:_____	RNAscope primary C4:_____	RNAscope primary C5:_____	RNAscope primary C6:_____
RNA targets using RNAscope probes designed to any open channel.	RNAscope probe C1:_____	RNAscope probe C2:_____	RNAscope probe C3:_____	RNAscope probe C4:_____	RNAscope probe C5:_____	RNAscope probe C6:_____

Order Assay Reagents

Assay Reagents	ACD Catalog number
☐ RNAscope Multiomic CORE & C1-C6 Channel Kit <i>Suitable for detection of up to 6 targets (RNA and/or protein targets).</i>	323175 (60 slides) 323465 (20 slides)
☐ RNAscope Multiomic CORE & C1-C3 Channel Kit <i>Suitable for detection of up to 3 targets (RNA and/or protein targets).</i>	323155 (60 slides) 323460 (20 slides)
<i>Individual C4-C6 channel items add additional channel capability to the CORE & C1-C3 channel kit.</i>	
☐ RNAscope Multiomic C4 Channel Reagents	322950 (60 slides) 323445 (20 slides)
☐ RNAscope Multiomic C5 Channel Reagents	322955 (60 slides) 323450 (20 slides)
☐ RNAscope Multiomic C6 Channel Reagents	322960 (60 slides) 323455 (20 slides)
<i>Special configurations for the ProximityScope Protein Assay.</i>	
☐ RNAscope™ Multiomic LS Protein Proximity Detection Kit-C6 <i>Includes the CORE and C6 channel reagents. For use with the ProximityScope antibodies. Enables detection of the proximity signal alone without other targets.</i>	322411 (60 slides) 322409 (20 slides)

Order Antibodies and Probes

User Provided Primary Antibodies

Order user provided rabbit or mouse antibodies if using RNAscope Secondary Antibodies or performing the ProximityScope Protein Assay. Note that the ProximityScope Protein Assay requires both a rabbit and a mouse hosted primary.

Item

- ☐ User provided primary antibody, rabbit host
- ☐ User provided primary antibody, mouse host

RNAscope Secondary Antibodies

Only required if detecting proteins with user provided primary antibodies. Note that secondary antibodies are available only in the listed channels.

Item	ACD Catalog number (60 slides)
☐ RNAscope anti-rabbit-C1	322954
☐ RNAscope anti-mouse-C2	322956

RNAscope Primary Antibody Panels

No secondary antibodies required. Note that panel antibodies are available only in the listed channels.

RNAscope Human Tumor Infiltrating Lymphocyte (TIL) Antibodies	ACD Catalog number (20 slides)
☐ RNAscope Ab Hs CD4-C3	322949
☐ RNAscope Ab Hs CD8-C4	322951
☐ RNAscope Ab Hs PanCK-C5	322952
☐ RNAscope Ab Hs FoxP3-C6	322953
☐ Human Tumor Infiltrating Lymphocyte (TIL) Antibody Panel Pack <i>Includes all four human TIL panel antibodies</i>	323185

RNAscope Neural Antibodies <i>Cross-reacts with mouse, human, and non-human primate proteins.</i>	ACD Catalog number (20 slides)
☐ RNAscope Ab NeuN-C3	AB0014-C3
☐ RNAscope Ab GFAP-C4	AB0024-C4
☐ RNAscope Ab IBA-1-C5	AB0034-C5
☐ RNAscope Multiomic™ Neural Antibody Panel Pack <i>Includes all three neuronal primary antibodies</i>	321780

ProximityScope Antibodies

Required for performing the ProximityScope Protein Assay.

Item	ACD Catalog number (20 slides)
☐ ProximityScope secondary antibodies-rabbit and mouse <i>Formerly RNAscope Protein Proximity secondary antibodies-rabbit and mouse. (Designed for the C6 channel. Requires user provided rabbit and mouse primary antibodies.)</i>	322415
☐ ProximityScope primary antibodies Human PD-1/PD-L1 <i>Formerly RNAscope Protein Proximity antibodies-Human PD1/PD-L1. (Designed for the C6 channel. Does not require use of secondary antibodies.)</i>	322405

RNA Target Probes

Order probes in appropriate channels. Probes can be selected in any open channel.

Probes	ACD Catalog number
RNAscope LS Target Probes	
☐ RNAscope LS Target Probes – [species] – [gene] -C1	
☐ RNAscope LS Target Probes – [species] – [gene] -C2	
☐ RNAscope LS Target Probes – [species] – [gene] -C3	
☐ RNAscope LS Target Probes – [species] – [gene] -C4	
☐ RNAscope LS Target Probes – [species] – [gene] -C5	
☐ RNAscope LS Target Probes – [species] – [gene] -C6	
☐ RNAscope Multiomic LS 6-plex Positive Control Probes- Hs <i>Control probes to other species also available.</i>	323198
☐ RNAscope Multiomic LS Assay - Positive Control Probes-Mm	322988
☐ RNAscope Multiomic LS 6-plex Negative Control Probes	323208
☐ RNAscope 2.5 LS Blank Probe diluent — required if not using a C1 probe <i>Included if using a C1 RNAscope probe but required as a separate item if not using a C1 probe. Approximately 500uL per probe per slide is required, plus container dead volume.</i>	300048

Order TSA Fluorophores

If using Vivid TSA Fluorophores for 3-plex assays

Item	ACD Catalog number	3-plex
☐ TSA Vivid Fluorophore 520	323271	✓
☐ TSA Vivid Fluorophore 570	323272	✓
☐ TSA Vivid Fluorophore 650	323273	✓

If using Akoya Biosciences TSA Fluorophores for assays up to 6-plex

Item	Akoya Biosciences Catalog number	3-plex	4-plex	5 or 6-plex
☐ Opal 480 Reagent Pack	FP1500001KT			✓
☐ Opal 520 Reagent Pack	FP1487001KT	✓	✓	✓
☐ Opal 570 Reagent Pack	FP1488001KT	✓	✓	✓
☐ Opal 620 Reagent Pack	FP1495001KT			✓
☐ Opal 690 Reagent Pack	FP1497001KT	✓	✓	✓
☐ Opal Polaris 780 Reagent Pack	FP1501001KT			
☐ Antibody Diluent/Block (needed when using Opal Polaris 780)	ARD1001EA		✓	✓

Note: For 5-plex assay, select any 5 of the recommended fluorophores to match your imaging capability.

Order Materials from Leica Biosystems

Instrument/Software

☐ Leica Biosystems' BOND RX System — automated slide stainer

☐ Software Version 6.0 or greater

Consumables

Item	Notes	Leica Biosciences Catalog number
☐ BOND 30 mL Open containers	QTY ~2 packs of 10 (including those on Research Detection System)	OP309700
☐ BOND 6 mL Titration kit (10 pack)	QTY 2 packs; 14 needed for training; 1/fluorophore except 780	OPT9049
☐ BOND Research Detection System	New and unregistered detection system required	DS9455/DS9777
☐ BOND Universal Cover tile		S21.4611
☐ BOND Epitope Retrieval Solution 1-1L (RTU)	Not used, but may need on deck depending on software version	AR9961
☐ BOND Epitope Retrieval Solution 2-1L (RTU)	Used for HIER protocols	AR9640
☐ BOND Dewax Solution – 1L (RTU)		AR9222
☐ BOND Wash Solution 10X Concentrate – 1L		AR9590
☐ BOND Aspirating Probe Cleaning System		CS9100
☐ BOND Mixing Stations		S21.1971

Other Required Materials

Item	Vendor	Catalog number
☐ Salmon Sperm DNA, sheared (10mg/ml)	ThermoFisher	AM9680
☐ Neutral Buffered Formalin solution, 10%	Sigma-Aldrich	HT5012-1CS
☐ Prolong Gold Antifade Mountant	ThermoFisher	P36930
☐ Mouse IgG2A Isotype Control	R&D Systems	MAB003
☐ Normal Rabbit IgG Control	R&D Systems	MAB1050

Kit Components and Storage

Kit/Box	Component	60-slide kit Volume/Amount	20-slide kit Volume/Amount	Storage Temp
RNAscope Multiomic LS CORE Reagents Cat No.: 60-slide kit: 322930 20-slide kit: 323425	RNAscope Multiomic LS AMP 1	21 mL x 1 bottle	8 mL x 1 bottle	2-8°C
	RNAscope Multiomic LS AMP 2	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic LS AMP 3	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic LS Hydrogen Peroxide	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic LS Rinse	29 mL x 3 bottles	11 mL x 2 bottle	
	RNAscope Multiomic LS DAPI	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope 2.5 LS Protease III	21 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope PretreatPro™	28mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic Antibody Diluent	29mL x 3 bottles	14 mL x 3 bottle	
RNAscope Multiomic Channel Reagents, C1-C6 Cat No.: 60-slide kit: 322935, 322940, 322945, 322950, 322955, 322960 20-slide kit: 323430, 323435, 323440, 323445, 323450, 323455	RNAscope Multiomic LS HRP	21 mL x 1 bottle	8 mL x 1 bottle	2-8°C
	RNAscope Multiomic TSA Buffer	29 mL x 1 bottle	8 mL x 1 bottle	
	RNAscope Multiomic HRP Blocker	29 mL x 1 bottle	8 mL x 1 bottle	
RNAscope and ProximityScope antibodies	-	-	-	Consult product label

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