

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

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.

ASSAY DESIGN // 01

Use this table to plan the channels needed for our assay based on your targets. Note that antibodies are available only in the listed channels. Probes can be designed to any open channel.

Target	RNAscope channel					
	C1	C2	C3	C4	C5	C6
Protein targets	User provided primary antibody-rabbit + RNAscope anti-rabbit-C1	User provided primary antibody-mouse + RNAscope anti-mouse-C2	-	-	-	-
	-	-	RNAscope Ab Hs CD4 -C3	RNAscope Ab Hs CD8 -C4	RNAscope Ab Hs PanCK-C5	RNAscope Ab Hs FoxP3-C6
RNA targets	RNAscope C1 probe	RNAscope C2 probe	RNAscope C3 probe	RNAscope C4probe	RNAscope C5 probe	RNAscope C6 probe

Order Assay Reagents

Assay Reagents	ACD Catalog number
<input type="checkbox"/> RNAscope Multiomic CORE & C1-C6 Channel Kit <i>Suitable for detection of up to 6 targets (RNA and/or protein targets)</i>	323175
<input type="checkbox"/> RNAscope Multiomic CORE & C1-C3 Channel Kit <i>Suitable for detection of up to 3 targets (RNA and/or protein targets).</i>	323155
Individual C4-C6 channel items add additional channel capability to the CORE & C1-C3 channel kit.	
RNAscope Multiomic C4 Channel Reagents	322950
RNAscope Multiomic C5 Channel Reagents	322955
RNAscope Multiomic C6 Channel Reagents	322960

Order Antibodies and Probes

User Provided Primary Antibodies

If using, must be paired with RNAscope Secondary Antibodies.

Item
<input type="checkbox"/> User provided primary antibody, rabbit host
<input type="checkbox"/> 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
<input type="checkbox"/> RNAscope anti-rabbit-C1	322954
<input type="checkbox"/> RNAscope anti-mouse-C2	322956

RNAscope Human Tumor Infiltrating Lymphocyte (TIL) Panel Antibodies

Optional, no secondary antibodies required. Note that panel antibodies are available only in the listed channels.

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

RNA Target Probes

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

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

Order TSA Fluorophores

If using Vivid TSA Fluorophores for 3-plex assays

Item	ACD Catalog number	3-plex
<input type="checkbox"/> TSA Vivid Fluorophore 520	323271	✓
<input type="checkbox"/> TSA Vivid Fluorophore 570	323272	✓
<input type="checkbox"/> 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
<input type="checkbox"/> Opal 480 Reagent Pack	FP1500001KT			✓
<input type="checkbox"/> Opal 520 Reagent Pack	FP1487001KT	✓	✓	✓
<input type="checkbox"/> Opal 570 Reagent Pack	FP1488001KT	✓	✓	✓
<input type="checkbox"/> Opal 620 Reagent Pack	FP1495001KT			✓
<input type="checkbox"/> Opal 690 Reagent Pack	FP1497001KT	✓	✓	✓
<input type="checkbox"/> Opal Polaris 780 Reagent Pack	FP1501001KT		✓	✓
<input type="checkbox"/> 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
<input type="checkbox"/> BOND 30 mL Open containers	QTY ~2 packs of 10 (including those on Research Detection System)	OP309700
<input type="checkbox"/> BOND 6 mL Titration kit	QTY ~76; 1/fluorophore except 780	OPT9049
<input type="checkbox"/> BOND Research Detection System (2)		DS9455/DS9777
<input type="checkbox"/> BOND Universal Cover tile		S21.4611
<input type="checkbox"/> BOND Epitope Retrieval Solution 1-1L (RTU)	Not used, but may need on deck depending on software version	AR9961
<input type="checkbox"/> BOND Epitope Retrieval Solution 2-1L (RTU)	Used for HIER protocols	AR9640
<input type="checkbox"/> BOND Dewax Solution – 1L (RTU)		AR9222
<input type="checkbox"/> BOND Wash Solution 10X Concentrate – 1L		AR9590
<input type="checkbox"/> BOND Aspirating Probe Cleaning System		CS9100
<input type="checkbox"/> BOND Mixing Stations		S21.1971

Other Required Materials

Item	Vendor	Catalog number
<input type="checkbox"/> Salmon Sperm DNA, sheared (10mg/ml)	ThermoFisher	AM9680
<input type="checkbox"/> Neutral Buffered Formalin solution, 10%	Sigma-Aldrich	HT5012-1CS

Kit Components and Storage

Kit/Box	Component	Volume/Amount	Storage Temp
RNAscope Multiomic LS CORE Reagents Cat No: 322930	RNAscope Multiomic LS AMP 1	21 mL x 1 bottle	2-8°C
	RNAscope Multiomic LS AMP 2	21 mL x 1 bottle	
	RNAscope Multiomic LS AMP 3	21 mL x 1 bottle	
	RNAscope Multiomic LS Hydrogen Peroxide	21 mL x 1 bottle	
	RNAscope Multiomic LS Rinse	29 mL x 3 bottles	
	RNAscope Multiomic LS DAPI	21 mL x 1 bottle	
	RNAscope 2.5 LS Protease III	21 mL x 1 bottle	
	RNAscope PretreatPro™	28mL x 1 bottle	
	RNAscope Multiomic Antibody Diluent	29mL x 3 bottles	
RNAscope Multiomic Channel Reagents, C1-C6 Cat No: 322935, 322940, 322945, 322950, 322955, 322960	RNAscope Multiomic LS HRP	21 mL x 1 bottle	2-8°C
	RNAscope Multiomic TSA Buffer	29 mL x 1 bottle	
	RNAscope Multiomic HRP Blocker	29 mL x 1 bottle	
RNAscope Ab Hs CD4-C3 Cat No: 322949	RNAscope Ab Hs CD4-C3	104 uL x 1 tube	2-8°C
RNAscope Ab Hs CD8-C4 Cat No: 322951	RNAscope Ab Hs CD8-C4	104 uL x 1 tube	2-8°C
RNAscope Ab Hs PanCK-C5 Cat No: 322952	RNAscope Ab Hs PanCK-C5	104 uL x 1 tube	2-8°C
RNAscope Ab Hs FoxP3-C6 Cat No: 322953	RNAscope Ab Hs FoxP3-C6	104 uL x 1 tube	2-8°C
RNAscope anti-rabbit-C1 Cat No: 322954	RNAscope anti-rabbit-C1	930 uL x 1 tube	2-8°C
RNAscope anti-mouse-C2 Cat No: 322956	RNAscope anti-mouse-C2	930 uL x 1 tube	2-8°C



Global Developer, Manufacturer, and Supplier of High-Quality Reagents, Analytical Instruments, and Precision Diagnostics.

INCLUDES R&D Systems™ Novus Biologicals™ Tocris Bioscience™ ProteinSimple™ ACD™ ExosomeDx™ Asuragen™ Lunaphore™

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