Quick Reference Guide

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ViewRNA™ ISH Tissue 1-Plex Assay

IMPORTANT: **If running ViewRNA ISH Tissue 1-Plex Assay for the first time, please refer to the *ViewRNA ISH Tissue 1-Plex Assay User Manual* to review assay guidelines and detailed procedures.**

Part 1: Sample Preparation and Target Probe Hybridization

Step		Task	
1	**Bake Slides** **65 min**	▪ Label slides with pencil ▪ Bake slides at 60 ± 1 °C for 60 min	
2	**Preparation for Part 1**	▪ Verify hybridization system is set to 40 ± 1 °C and appropri ately humidified ▪ Prepare: ▫ 2 L 1X PBS ▫ 200 mL 10% NBF in 1X PBS ▫ 3 L Wash Buffer ▫ 500 mL 1X Pretreatment Solution ▫ 200 mL Storage Buffer (optional) ▫ 1 L ▪ Ensure availability of: ▫ 400 mL 100% ethanol ▫ 1.4 L ddH_2O ▫ 600 mL xylene or 400 mL HistoClear ▫ 200 mL Gill's Hematoxylin I ▫ 200 mL of 3 µg/mL DAPI in 1X PBS (optional, for fluorescence detection) ▪ Thaw probe set(s) and place on ice ▪ Prewarm 40 mL 1X PBS and Probe Set Diluent QF to 40 °C	▪ Optional for 1-day assay: ▫ Prewarm Amplifier Diluent QF and Label Probe Diluent QF to 40 °C ▫ Thaw PreAmp 1 QF and Amp1 QF, Mix, briefly spin down to collect contents, and place on ice until use ▫ Briefly spin down the Label Probe-AP and place on ice ▫ Bring Fast Red Tablets, Napthol Buffer, and AP Enhancer Solution to RT ▫ Prepare 1 L 0.01% ammonium hydroxide under a fume hood
3	**Deparaffinization** **30 min**	If using xylene (work in a fume hood): ▪ Incubate slides 3 times, each for 5 min in xylene ▪ Wash slides 2 times in 100% ethanol, 5 min each wash ▪ Decant 100% ethanol from slides and let air dry	If using Histo-Clear: ▪ Bake slides at 80 ± 1 °C for 3 min ▪ Incubate slides 2 times, 5 min each in HistoClear ▪ Wash slides 2 times in 100% ethanol, 5 min each wash ▪ Decant 100% ethanol from slides and let air dry
4	**Draw Hydrophobic Barrier** **40 min**	▪ Create hydrophobic barrier ▪ Allow slides to air dry at RT for 20-30 min ▪ Heat 1X Pretreatment Solution to 90-95 °C while slides are drying	
5	**Heat Pretreatment** **10-25 min, depending on optimized time**	▪ Heat slides in 1X Pretreatment Solution at 90-95 °C for the optimal time determined in the pretreatment assay optimization procedure ▪ Wash slides 2 times in ddH_2O, 1 min each wash ▪ Transfer slides to 1X PBS **IMPORTANT:** Do not let the slides dry out from this point forward.	
6	**Protease Digestion and Fixation** **30-50 min, depending on optimized time**	▪ Prepare 1:100 working protease solution in prewarmed 1X PBS ▪ Add working protease solution to slides ▪ Incubate at 40 °C for optimal time time determined in the pretreatment assay optimization procedure ▪ Wash slides 2 times in 1X PBS, 1 min each wash ▪ Fix slides in 10% NBF at RT for 5 min under a fume hood ▪ Wash slides 2 times in 1X PBS, 1 min each wash	

Step		Task
7	**Target Probe Set Hybridization** **3 hr 10 min**	▪ Prepare 1:50 working probe set solution in prewarmed Probe Set Diluent QF ▪ Add working probe set solution to slides ▪ Incubate at 40 °C for 3 hr
8	**Wash Slides** **8 min**	Wash slides 3 times in Wash Buffer, 2 min each wash
9	**Optional Stop Point** **1 min**	▪ Store slides in Storage Buffer at RT for up to 24 hr. Cover dish with lid or sealing film to prevent evaporation. ▪ Store remaining 1X PBS and Wash Buffer at RT for use in Part 2

Part 2: Signal Amplification and Detection

Step		Task
10	**Preparation for Part 2** **10 min**	▪ Pour Gill's Hematoxylin I into a clear staining dish, store at RT protected from light. ▪ Optional – Prepare 200 mL 3 µg/mL DAPI, store at 4 °C until use or place on ice ▪ Prewarm Amplifier Diluent QF and Label Probe Diluent QF to 40 °C ▪ Thaw PreAmp1 QF and Amp1 QF, place on ice ▪ Bring Fast Red Tablets, Naphthol Buffer, and AP Enhancer to RT ▪ Prepare 1 L 0.01% ammonium hydroxide
11	**Wash Slides** **8 min**	Remove slides from Storage Buffer and wash 3 times in Wash Buffer, 2 min each wash with constant, vigorous agitation
12	**PreAmplifier Hybridization** **35 min**	▪ Prepare 1:100 Working PreAmp1 Solution in prewarmed Amplifier Diluent QF ▪ Add working PreAmp1 solution to slides ▪ Incubate at 40 °C for 25 min
13	**Wash Slides** **8 min**	Wash slides 3 times in Wash Buffer, 2 min each wash with constant, vigorous agitation
14	**Amplifier Hybridization** **20 min**	▪ Prepare 1:100 working Amp1 solution in prewarmed Amplifier Diluent QF ▪ Add working Amp1 solution to slides ▪ Incubate at 40 °C for 15 min
15	**Wash Slides** **8 min**	Wash slides 3 times in Wash Buffer, 2 min each wash
16	**Label Probe-AP Hybridization** **20 min**	▪ Prepare 1:1000 working Label Probe-AP solution in prewarmed Label Probe Diluent QF ▪ Add working Label Probe-AP solution to slides ▪ Incubate at 40 °C for 15 min
17	**Wash Slides** **12 min**	Wash slides 3 times in Wash Buffer, 3 min each wash
18	**Apply Fast Red Substrate** **45 min**	▪ Add AP-Enhancer to slides and incubate at RT for 5 min ▪ Prepare Fast Red Substrate (1 Fast Red Tablet/5 mL Napthol Buffer) ▪ Decant AP-Enhancer and add Fast Red Substrate to slides, incubate at 40 °C for 30 min ▪ Rinse slides in 1X PBS for 1 min
19	**Counterstain** **25 min**	▪ Incubate slides in Gill's Hematoxylin I stain at RT for 5-10 sec ▪ Wash slides 3 times in ddH_2O, 1 min each wash ▪ Incubate in 0.01% ammonium hydroxide at RT for 10 sec ▪ Wash slides in ddH_2O for 1 min ▪ Optional – Incubate slides in DAPI at RT for 1 min, wash slides in ddH_2O for 1 min ▪ Let slides completely air dry at RT (~20 min)

Step		Task	
20	**Mount and Image** **40 min**	**DAKO Ultramount:** **For no coverslipping** ▪ Add Ultramount to tissue sections ▪ Place slides in a 70 °C oven/incubator for 10-30 min ▪ Observe under brightfield or fluorescence microscope ▪ Store slides at RT **For post mounting with coverslip** ▪ Work under a fume hood and follow the no coverslip procedure ▪ Allow the slides to come to RT ▪ Apply Histomount directly on top of the dried Ultramount ▪ Place coverslip ▪ Air dry at RT for 15 min ▪ Observe under brightfield or fluorescence microscope ▪ Store slides at RT	**Innovex Advantage Mounting Media:** ▪ Add Advantage Mounting Media to cover glass and invert tissue slide to cover ▪ Flip over, allow slides to dry ▪ Seal all four edges with nail polish ▪ Observe under brightfield or fluorescence microscope ▪ Store slides at RT

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