

You are here: [Slide Prep](#) > RNAscope & DSP combined protocol

RNAscope & DSP combined protocol

Day 1

1. Refer to the **RNAscope LS Multiplex Fluorescent Reagent Kit** or the **RNAscope Multiplex Fluorescent Kit V2 User Manual** provided by Advanced Cell Diagnostics for an overview of guidelines and protocols and materials required to run the RNAscope assay.
2. If using the automated RNAscope assay, DAPI will not be used for DNA staining. Register a new 30 mL BOND container for DAPI and fill it with DEPC-treated water. This will allow the Leica to run the protocol without having your slides get stained with DAPI. Label/indicate on the container that it is a “fake” DAPI container and replace it with the real DAPI container on the reagent tray.
3. Proceed with running the assay on the Leica Biosystems BOND RX Research Advanced Staining System with the following recommended TSA fluorophore dilutions ([see Table 32](#)).
 - If using the RNAscope manual assay, proceed through the HRP signal development steps (page 34 of the **RNAscope Multiplex Fluorescent Kit V2 User Manual**).

Table 32: TSA fluorophore dilutions

Fluorescence	Product number (PerkinElmer)	Recommended dilution
PerkinElmer TSA Plus Cyanine 3 System	NEL744001KT	1:1500
PerkinElmer TSA Plus Cyanine 5 System	NEL745001KT	1:3000



NOTE: Dilution of TSA fluorophores may need to be optimized for different targets and certain tissue types.

4. The total run time for the assay is 10–14 hours depending on the number of fluorophores and slides. Ideally, the Leica run will be set-up in the afternoon and will run overnight.

Day 2

1. Upon completion of the Leica run, place your slides in deionized water.

2. Refer to relevant sections of the **GeoMx DSP Slide Prep Manual** for an overview of guidelines and protocols and materials required to run the DSP assay. Begin the RNA slide preparation at step 6, [Postfix —Preserve tissue morphology for soft tissues \(20 min\)](#).
3. Proceed with step 7, [Manual RNA Slide Preparation Protocol](#).

Day 3

1. Proceed with step 8, [Perform stringent washes to remove off-target probes \(90 minutes\)](#).
2. Perform DNA staining with SYTO 13 provided by NanoString and wash two times with 2X SSC.
3. Load your slides on DSP.