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Adding custom antibodies during slide prep

1. Follow the GeoMx DSP instrument [Manual FFPE Protein Slide Preparation Protocol](#) up to [Primary antibody incubation \(Overnight\)](#).
2. Custom-labeled GeoMx antibodies will arrive at a concentration of either **200 µg/mL** (from NanoString) or **50 µg/mL** (from [Abcam](#)), depending on the provider. Antibodies should be stored at 4°C for use within two weeks or aliquoted and kept at -80°C for longer term storage.
3. The following recommendations are for the staining of one slide with a custom antibody concentration of **0.25 µg/mL**. The optimal concentration of custom antibody can be determined by the user.
 - a. For NanoString antibodies, **dilute 200 µg/mL custom antibody to 50 µg/mL** by **adding 2 µL of custom antibody to 6 µL of Buffer W**. Pipet up and down at least 10 times to mix thoroughly.
 - b. **Add 1.1 µL of diluted custom antibody** to the working antibody solution prepared in [Primary antibody incubation \(Overnight\)](#) of the Manual FFPE Protein Slide Preparation Protocol.

Preparing Abcam Probe R Master Stock for nCounter Readout

Abcam provides a Probe R tube for each antibody to be used. Prior to making the Probe R Working Pool (see [Create Probe R and Probe U Working Pools](#)), individual Probe Rs must be combined to make a Probe R Master Stock.

1. **Thaw the Probe R tubes** corresponding to the antibodies used in Slide Preparation
2. **Pipet 2 µL** of each Abcam Probe R into a fresh tube.
3. **Add nuclease-free water** for a total volume of **10 µL**.
4. Add the volume of Probe R Working Pool as indicated in the Probe R working pool dilutions table ([see Table 38](#)).