For Proseek Multiplex with product number 94600 and 94350 a sample dilution step is required prior to running the assay protocol. Please read the entire Proseek Multiplex manual.

1. BEFORE STARTING
   - Decide how many samples you will include in the experiment and the number of replicates.
   - Use the 96-well plate layout below and select a location for each sample. Make sure the samples are distributed randomly. **Note:** The negative control and interplate control samples should not be diluted.
   - Sample dilutions should be made in a 96-well plate (0.2 mL per well) using a multi-channel pipette. **Note:** Pipette from the upper most part of the samples to prevent liquid from sticking to the outside of the pipette tip.
   - Prepare the dilutions freshly, as close to the start time of the assay as possible.

![Dilution Plate layout](image)

**Fig 1.** Dilution Plate layout.

2. SAMPLE DILUTION
   - Thaw the Sample Diluent (Art. No 84032), vortex and empty the bottle into a multi-channel pipette reservoir (minimum volume, 15 mL).
   - Transfer 99 µL of the Sample Diluent into each well of columns 1-11 and positions A-B in column 12 by using reverse pipetting into a 96-well PCR plate. **Note:** Pipette the Sample Diluent carefully to avoid foaming. Do not change pipette tips and use the same multi-channel pipette throughout the entire dilution series. Name this plate Dilution Plate.
Carefully transfer 1 µL of your samples according to your plate layout, see Figure 1. **Note:** Use the same multi-channel pipette throughout the entire plate, also for samples A-B of column 12. Change tips between each pipetting step.

- Seal the plate with adhesive plastic film.
- Vortex the plate thoroughly and ensure that all wells are mixed. Spin down the contents at 400 × g for 1 min at room temperature.
- Proceed with the protocol according to the Proseek Multiplex User Manual, see section 5.6 for instructions.