

Proseek[®] Multiplex

SAMPLE DILUTION GUIDELINES (1:2025)

For Proseek Multiplex with product number 94360, a 2-step sample dilution (1:2025) is required prior to running the assay protocol. Please read the entire Proseek Multiplex manual before starting.

BEFORE STARTING

- ▶ Decide how many samples you will include in the experiment and the number of replicates (up to 90 samples). Please note that you will need a double set of consumables.
- ▶ A 2-step sample dilution should be performed in order to reach the desired dilution factor of 1:2025, and made in 2×96 -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.
- ▶ Use the 96-well plate layout below and select a location for each sample. Make sure the samples are distributed randomly. **Note:** The 3 negative control and 3 interplate control samples should not be diluted.
- ▶ Prepare the dilutions freshly, as close to the start time of the assay as possible.

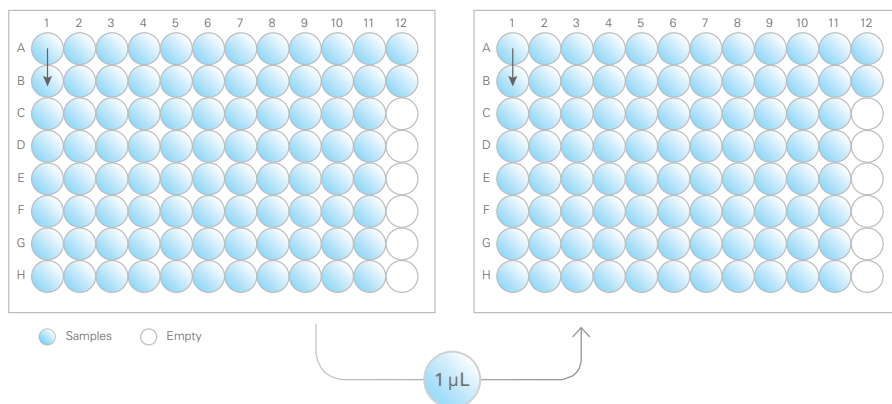


Fig 1. Dilution plate 1 and 2 (1:44 + 1:44).

SAMPLE DILUTION

1. Thaw the Sample Diluent (Art. No 84032), vortex and empty the bottle into a multi-channel pipette reservoir (minimum volume, 15 mL).
2. Mark two 96-well plates. Transfer 44 μL of the Sample Diluent into each well of columns 1–11 and positions A–B in column 12 by using reverse pipetting. Do not change pipette tips and use the same multi-channel pipette throughout the entire dilution series. Repeat the same procedure for both plates. **Note:** *Pipette the Sample Diluent carefully to avoid foaming.* Name the plates *Dilution Plate 1* and *2*.
3. Carefully transfer 1 μL of your samples into *Dilution plate 1* 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.*
4. Seal the plate with adhesive plastic film.
5. Vortex the plate thoroughly and ensure that all wells are mixed. Spin down the contents at $400 \times g$ for 1 min at room temperature.
6. Transfer 1 μL of your diluted samples from *Dilution plate 1* to previously prepared *Dilution plate 2* (see step 2). Remember to change tips in between.
7. Seal the plate with adhesive plastic film.
8. Vortex the plate thoroughly and ensure that all wells are mixed. Spin down the contents at $400 \times g$ for 1 min at room temperature.
9. Proceed with the protocol according to the Proseek Multiplex^{36x96} User Manual using *Dilution plate 2*, see section 5.6 for instructions. **Note:** *Diluted samples should not be saved for further analysis.*

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