Mercodia

Technical Note, No: 34-0164, v.1.0

25 µL sample volume in the Oxidized LDL ELISA

The Oxidized LDL ELISA (10-1143-01) is validated with a sample volume of 10 µL, but it is still possible to choose to use 25 µL sample for dilution.

Sample volume

The updated Oxidized LDL (OxLDL) ELISA (10-1143-01, relaunched 1st of September 2021) is validated with a sample volume of 10 µl. Mercodia, therefore, recommends that this sample volume is used in the dilution process. It is, however, possible to choose to use a sample volume of 25 µL. Note that by doing this, Mercodia can only recommend that the assay is used for research (**Research Use Only**) and not for diagnostic procedures.

The recommended dilution protocol is found in the Directions for Use (DfU, 31-3136, from v. 23.0), and the alternative dilution protocol using 25 µL sample is described in detail below and illustrated in figure 1.

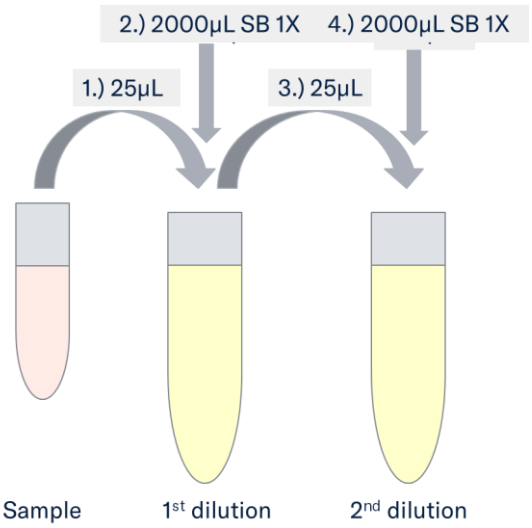

Figure 1: Dilution process using 25 µL sample. 1.) 25 µL sample is pipetted into an empty tube, 2.) 2000 µL Sample Buffer (SB) 1X is added to the tube, 3.) 25 µL of the first dilution is added to a second tube, 4.) 2000 µL SB1X I added to the second tube.

Alternative dilution protocol (Research Use Only)– dilution of samples using 25 µL sample

The dilution of samples is a very important step in the assay procedure. If the dilution is not properly performed there is a risk of increased variation in measured oxidized LDL concentration. Each sample must be diluted in two steps for a final dilution of 1/6561. Samples may be thawed in room temperature or on ice. Avoid keeping undiluted samples in room temperature for more than one hour.

1. Prepare sample buffer 1X solution by diluting one bottle Sample Buffer 4X* (50 mL) in 150 mL redistilled water. Mix approximately 15 minutes using a magnetic stirrer to ensure a homogenous solution.
2. Add 25 µL of each sample to individual tubes.
3. Add 2000 µL sample buffer 1X solution to each tube for a 1/81 dilution.
4. Cap all tubes of the first dilution and mix thoroughly using a vortex mixer and by inverting the tubes.
5. Add 25 µL of each 1/81 dilution to new individual tubes.
6. Add 2000 µL sample buffer 1X solution to each tube for a final dilution of 1/6561.
7. Cap all tubes of the second dilution and mix thoroughly using a vortex mixer and by inverting the tubes.
8. Let each final sample dilution sit on the bench for 10 minutes and then mix again before the samples are added to the plate. The assay should be started within one hour of dilution and the diluted samples should not be stored.

**Note: A precipitate may form in the Sample Buffer 4X when stored at 2-8°C. Allow the buffer to reach room temperature and mix until the precipitate has dissolved before diluting the concentrate in redistilled water.*