Bioxol Fejlesztő Gyártó és Kereskedelmi Kft.
1155 Budapest Wysocki utca 1.

Instruction for use

Lipid Detection Kit (LD-1012)

Kit contents:

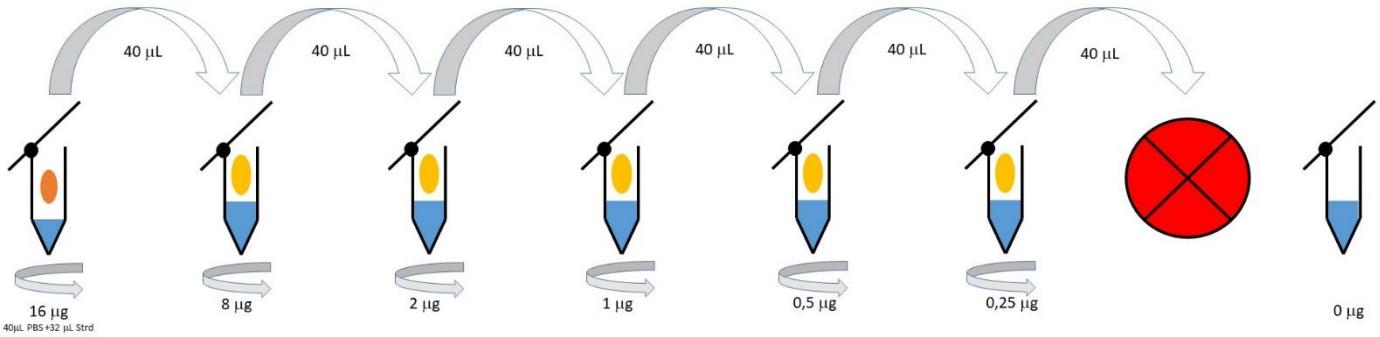
1. **LD-2012** Bio-Lipid Standard (1 mg/mL PBS based emulsion)
2. **LD-3012** Bio-FV Reagent

Required tools and chemicals that the kit does not include:

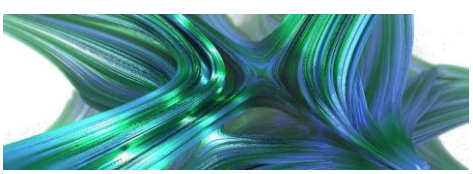
1. 1.5 mL well-sealed, surface-treated-free and dye-free microcentrifuge tubes. (Recommended microcentrifuge tubes: Safe-Lock tubes, 1.5 mL, 0030 120.086 és PCR clean tubes, 1,5 mL 0030 125.215, Eppendorf AG, Germany). In case of centrifuge tube from alternative supplier, a test measurement is recommended. If the background (in case of lipid-free sample) is bigger than 0.20 arb measured at 540 nm, the microcentrifuge tube can not be used during the assay.
2. Pipette tips
3. Micropipette suitable for pipetting 96% sulfuric acid
4. Device suitable for reading a 96-well plate and measuring absorbance at 540 nm wavelength, a belonging plate with a volume at least 300 µL per well
5. Thermoblock suitable for heating microcentrifuge tubes at 90 °C
6. Incubator that provides 37 °C
7. Chemical extraction cabinet
8. 96% sulfuric acid
9. PBS or other buffer free from substances (e.g. sugars) oxidizable by sulfuric acid. E.g. NaCl-Hepes or high purity water

Sample preparation

1. Prepare the sample in a volume of 40 µL, and the PBS in a 1,5 mL microcentrifuge tube.
2. Using a two-fold dilution (40 µL final volume), prepare a standard series (16-8-4-2-1-0,5-0,25-0 µg lipid) using the Bio-Lipid standard.
    a. In case of a 16 µg standard, add 32 µL Bio-Lipid standard to 48 µL PBS and vortex it.
    b. Take 40 µL of the emulsion and add it to 40 µL PBS in another tube. Vortex it, and repeat the steps until reaching the concentration of 0,25 µg. In the final step, throw out the removed 40 µL emulsion. To examine the background (blank – 0 µg) use 40 µL of PBS. If the samples are not dispersed in PBS, prepare a dilution series with the sample's buffer.



3. Add 200µL 96% sulfuric acid to the 40µL sapmle/standard solution, homogenizate with vortex and incubate it in an extraction cabinet with opened lid for 20 minutes at 90°C



4. Close the lid of the microcentrifuge tubes after the incubation period and cool the mixture for 5 minutes at 4°C.
5. Add 120 µL Bio-FV reagent to the cooled reaction mixture, homogenizate with vortex, then put 280 µL from the mixture in a 96-well plate.
6. Incubate the plate for 1 hour at 37°C
7. Measure the absorbance of the colourful (pink) reaction product at 540 nm.
8. Calculate the lipid content of the samples based on the slope of the standard curve. The standard curve can be used only if the value of R^2 of the fitted line is higher than 0.95. The result below 0,5 µg lipid is indicative (LoD ~0,2 µg) and LoQ ~0,5µg lipid.