Metabolic parameters determination in acid citrated plasma

Bianca Hemmeryckx,Dries Bauters,H. Roger Lijnen

Abstract

Diet-induced obesity by administration of a western-type diet high in fat and sugar to mice may result in an elevation of plasma total and LDL-cholesterol levels, triglycerides and the levels of the liver enzymes alkaline phosphatases and aspartate (AST) and alanine aminotransferases (ALT).

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Guidelines

-The acid citrate solution is best kept at +4°C and made fresh every month.

-Concentrations of plasma metabolic parameters need to be multiplied by the dilution factor 3.

Protocol

Step 1.

Collect 300µl blood from the retro-orbital sinus using a capillary tube containing acid citrate and collect the blood in an eppendorf tube containing 30µl acid citrate (1/10 dilution). Do not include the first two drops of blood.
Acid citrate (50 ml): dissolve 1.25g trisodiumcitrate dihydrate (85 mM) and 0.82g citric acid monohydrate (78 mM) in 20 ml milliQ water and add MilliQ water until volume is 50 ml. pH should be 4.5.

REAGENTS
Trisodium citrate dihydrate S1804 by Sigma-aldrich
citric acid monohydrate 33114 by Sigma-aldrich

Step 2.

Plasma is obtained by centrifuging the blood in an eppendorf centrifuge for 10 min at 10,000 rpm. Collect supernatant.

Step 3.

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Mix 50µl of acid citrated plasma with 100µl metabolic buffer consisting of 0.1 M Tris pH 7.5 and 0.15 M NaCl in a 1 ml sample cup provided ny the routine lab of UZ Leuven, Leuven, Belgium.

Step 4.

Transfer samples to the routine lab of UZ Leuven, Leuven, Belgium for the measurement of plasma levels of total cholesterol, LDL- and HDL-cholesterol, triglycerides, alkaline phosphatases and aspartate and alanine aminotransferases.

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