

# Hepatic VLDL secretion assay

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## Abstract

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## Protocol

### Step 1.

- Male C57BL/6 mice are fasted for 4 hours before initiation of VLDL secretion assay.

### Step 2.

- After baseline blood collection (50ul) from the retro-orbital sinus, mice are intraperitoneally injected with poloxamer-407 (P-407) (10% (w/v) in saline, Sigma-Aldrich, Cat No 16758) at a dose of 1.0 g/kg of body weight.

### Step 3.

- After 1, 2 and 5 hours, approximately 50ul of blood is collected from the retro-orbital sinus.

### Step 4.

- Blood collection and injections are conducted on mice sedated under isoflurane (inhalation with 1-4% in O<sub>2</sub>). We keep the mice in anesthesia before and during drug injection or bleeding procedure; and after confirmation of stopping of bleeding, we will put the mice back to the original cages to allow breathing under normal aerobic environment. We will repeat this anesthesia and bleeding procedure for each time of bleeding.

### Step 5.

- TG and apoB concentrations, as surrogates of VLDL, in plasma samples are measured by enzymatic quantification assay kits purchased from Wako Diagnostics and Western blotting, respectively.