# Lipoprotein Lipase Activity Assay (Fluorometric)

**Abcam**

## Abstract

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## Protocol

### Plasma

**Step 1.**

1. To measure maximum LPL activity in plasma, inject mouse/rat with 0.2 Units heparin/gram of body weight by tail vein injection.
2. Collect blood 10 minutes after injection.
3. Centrifuge samples at 3000 x g for 15 min. at 4 degree Celcius using a cold microcentrifuge.
4. Collect supernatant and transfer to a clean tube.
5. Initial recommendation for assay: 1-10 uL/well

### Assay Procedure

**Step 2.**

- Equilibrate all materials and prepared reagents to room temperature prior to use
- We recommend that you assay all standards, controls and samples in duplicate

1. Set up Reaction Wells
2. Standard wells = 50 µL standard dilutions.
3. Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with ddH2O).
4. Background Control wells= 50 µL ddH2O.
5. Positive Control wells = 4 µL diluted positive control (section 12.1) + 46 µL ddH2O.
6. Assay Control wells = 4 µL diluted positive control (section 12.1) + 2 µL Inhibitor + 44 µL ddH2O.

### LPL Reaction

**Step 3.**

- Prepare 50 µL of Reaction Mix for each standard reaction. Mix enough reagents for the number of standard reactions to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

| Components | Reaction Mix Standard (µL) |
|---|---|
| Positive Control | 4 |
| $ddH_2O$ | 46 |

- Add 50 µL of Reaction Mix into each standard well. Mix well.
- Add 50 µL of diluted Substrate (see Section 9.2) into each sample, Positive Control and assay validation well. Mix well

The table below summarizes how to set up the reactions:

| Components | Standard well (µL) | Sample well (µL) | Backgound control well (µL) | Positive control well (µL) | Assay Control well (µL) |
|---|---|---|---|---|---|
| Standard | 50 | - | - | - | - |
| Sample | - | 1 – 50 | - | - | - |
| Diluted positive control (1/100) | - | - | - | 4 | 4 |
| Inhibitor | - | - | - | - | 2 |
| $ddH_2O$ | - | Up to 50 | 50 | 46 | 44 |
| Reaction Mix Standard | 50 | - | - | - | - |
| Diluted substrate | - | 50 | 50 | 50 | 50 |
| **TOTAL WELL** | **100** | **100** | **100** | **100** | **100** |

- Pre-incubate the plate at 37°C for 10 minutes protected from light to stabilize the signal.

- Measure output at Ex/Em = 482/515 nm on a microplate reader in a kinetic mode, every 10 minutes, for at least 1 hour at 37°C protected from light.

NOTES

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Incubation time depends on the LPL Activity in the samples. We recommend measuring fluorescence in a kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the LPL activity of the samples. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).

RFU value at T2 should not exceed the highest RFU in the standard curve. For standard curve, do not subtract RFU1 from RFU2 reading.

## Calculations

**Step 4.**

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

- Ensure you are using the linear portion of the kinetic reading when calculating enzymatic activity.

13.1. Average the duplicate reading for each standard and sample.

13.2. Subtract the mean RFU value of the blank (Standard #1) from all standard and sample readings. This is the corrected RFU.

13.3. Plot the corrected RFU values for each standard as a function of the final concentration of Substrate.

13.4. Subtract Background Control (RC) reading from Sample (S) reading.

$$\mathbf{\Delta RFU_S = RFU_{2S} - RFU_{1S}}$$

$$\mathbf{\Delta RFU_{BC} = RFU_{2BC} - RFU_{1BC}}$$

$$\mathbf{Corrected\ RFU = RFU_S - RFU_{BC}}$$

13.5. Use the corrected $\Delta RFU_{482/515nm}$ to obtain B pmol of Substrate formed during the reaction time ($\Delta T = T2 - T1$).

13.6. Activity of LPL in the test samples is calculated as:

$$LPL\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D = pmol/mL/min = mU/mL$$

Where:

B = Amount of substrate in the sample well calculated from Standard Curve (pmol).

$\Delta T$ = Reaction time (min).

V = Original sample volume added into the reaction well (mL).

D = Sample dilution factor.

Alternatively, calculate the slope for all samples (S) and Reagent Control (RC) as follows:

13.7. Divide the net ΔRFU (RFU2 – RFU1) values of samples and background control by the time ΔT (T2 – T1).

13.8. Subtract the slope of Background Control (BC) from the slope of sample (S) to get the Sample corrected slope (S-corrected).

13.9. Activity of LPL in the test samples is calculated as:

$$LPL\ Activity\ (mU) = \left(\frac{Slope_{S-corrected}}{Slope_{Standard}}\right)$$

$$= \left(\Delta RFU_S/\Delta T_S - \Delta RFU_{BC}/\Delta T_{BC}\right)\left(\Delta RFU_C/pmol\right)$$

Where:

$Slope_{S\text{-}corrected}$ is the corrected slope of the sample.

$Slope_{Standard}$ is the slope of Standard Curve.

**Unit Definition:**

**1 Unit LPL activity =** amount of Lipoprotein Lipase that generates 1.0 nmol of fatty acid product per min. at pH 7.4 at 37°C.