Roar

Product Information

Roar MTP Activity Assay Kit, 100 assays

Catalog No. RB-MTP

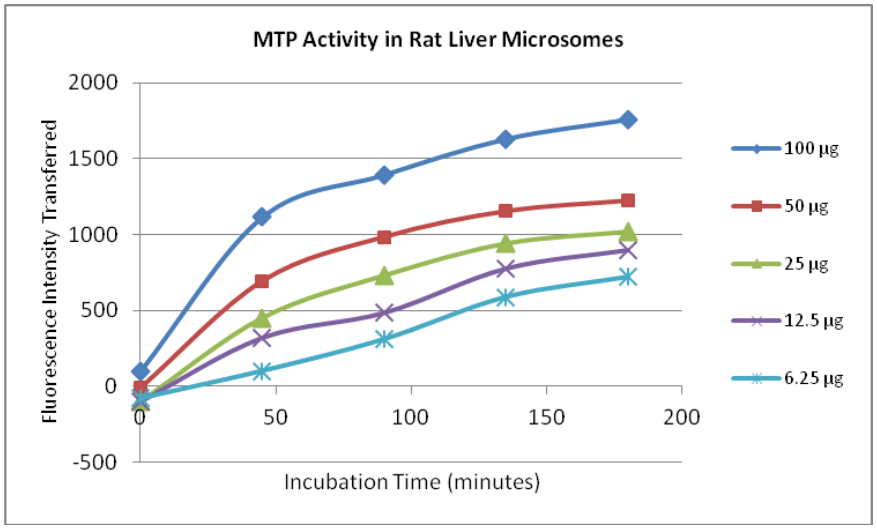
U.S. Pat. Nos. 5,585,235; 5,618,683; 5,770,355

Assay Method:	Fluorometric
Number of Assays:	100 assays in 200 µl total assay volume
Kit Contents:	Donor particle: 400 µl Acceptor particle: 400 µl Assay buffer: 20 ml
Storage and Handling:	Store kit components at 4°C. If stored properly, components are stable for up to 1 year. DO NOT FREEZE.
Instrumentation:	Fluorescence spectrophotometer Excitation: 465 nm / Emission: 535 nm.

Introduction

Microsomal triglyceride transfer protein (MTP), a membrane-bound protein present in the liver, plays an important role in the assembly and secretion of very low density lipoprotein (VLDL).

The Roar MTP Activity Assay Kit is a sensitive, homogeneous fluorometric assay useful for continuous measurement of MTP activity in cell lysate or tissue homogenate.



The Roar MTP Activity Assay Kit includes proprietary substrates that enable the detection of MTP-mediated transfer of neutral lipid. The transfer activity results in an increase in fluorescence intensity. DMSO tolerance is 10% (v/v) and the assay components are stable for up to one year.

Materials Required, But Not Supplied

Fluorimeter with appropriate wavelength capabilities (Ex: 465 nm; Em: 535 nm)
37°C water bath / incubator
Adhesive microplate sealers
MTP source: HepG2 cell homogenate or partially purified MTP (Roar #RB-HLV)
Homogenization buffer (for preparation of HepG2 cells): 150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.4, 0.5 mM PMSF and 20 µg/ml leupeptin.

Assay Method

1. The total assay volume per well should be 200 µl (4 µl donor + 4 µl acceptor + assay buffer + MTP source); add the donor particle, acceptor particle, and buffer before the MTP source, which should be delivered last. A master mix prepared with donor particle, acceptor particle, and buffer may first be distributed among all the wells of a plate, followed by the addition of the MTP source.

2. Prepare a blank that contains 4 µl of donor particle, 4 µl of acceptor particle, and 192 µl assay buffer.

3. Incubate for 3 - 6 hours (depending upon the specific activity of your MTP source) at 37°C.

4. Measure the increase in fluorescence of samples using a fluorimeter (excitation: 465 nm; emission: 535 nm). Determine the fluorescence intensity transferred in the samples by subtracting the blank fluorescence intensity from each sample.

Notes:

Sample volumes may need to be adjusted depending on the specific activity of the MTP source in your assay. For samples with low activity, decrease the buffer volume and increase the volume of your sample in the assay. Alternatively, microplates containing samples may be incubated overnight at 25°C. MTP remains active at 25°C.

A heat-killed sample may be prepared in sealed microtubes at 56°C for ~5 minutes to inactivate the protein. Cool the microtube and spin down any condensation.

Sigma supplies the Pfizer MTP inhibitor CP-346086 (Sigma #PZ0103) - see below for instructions on validating this assay with the inhibitor.

Assay Standardization

1. The concentration of the fluorescent substrate is printed on the label of the donor particle vial. The standard curve to derive the relationship between fluorescence intensity transferred and moles of substrate transferred is generated by dispersing the donor particle in isopropanol (IPA). Use spectrally pure isopropanol (HPLC grade or better) as the solvent. There should not be any background fluorescence when isopropanol alone is read.

2. Prepare six test tubes labeled ‘T0’ to ‘T5’ each containing 1ml isopropanol, adding an additional 1 ml of isopropanol to ‘T5’.

3. Pipette 5 µl donor particle to the test tube labeled ‘T5’, thoroughly mix (vortex) to adequately disperse the donor particle in the isopropanol.

4. Serially dilute from ‘T5’ to ‘T1’ and read the fluorescence intensity (EX 465 / EM 535) of the samples from tubes ‘T0’ to ‘T5’.

5. Perform a linear regression using the fluorescence intensity reads at each concentration of the standard curve and calculate the pmoles transferred in your assay.

Protocol for Preparing HepG2 Cell Homogenate

1. Grow HepG2 cells in 75 cm^2 T-flasks until confluent.

2. Suspend the cells from 6 flasks in a total of 5 ml of homogenization buffer (approximate protein concentration will be 10 mg/ml).

3. Sonicate the suspension on ice with five 5-second bursts in a 550W sonicator (power setting: 4) fitted with a microtip.

4. Use 100 µg of homogenate protein in the RB-MTP activity assay.

Homogenization Buffer

To 100 ml of 10mM Tris / 150 mM NaCl / 1mM EDTA, pH 7.4, add 0.5 ml of 100 mM PMSF (Sigma #P7626) in ethanol and 2 ml of 1mg/ml leupeptin (Sigma #L2884) in Tris-saline.

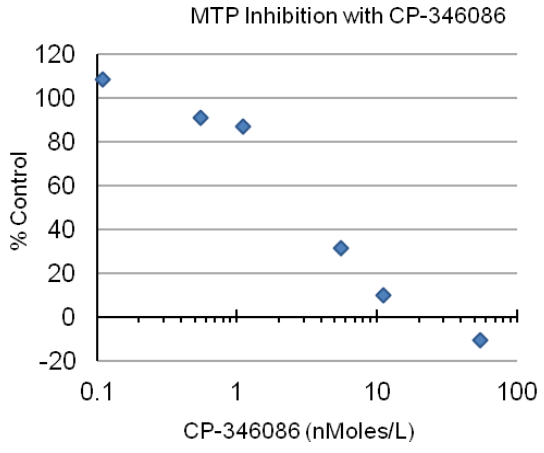
Notes: It is not necessary to spin down cell debris to make a low-speed supernatant, nor is it necessary to partially purify membranes to assay MTP activity.

Assay Validation with MTP Inhibitor CP-346086 (Sigma #PZ0103)

Purified MTP must be used with CP-346086. In the protocol below, MTP was purified from rat liver microsomes.

Prepare: 50 ml 100 mM tris / 100 mM KCl / 10 mM $MgCl_2$ / pH 7.4
0.54% deoxycholate in 50 mM tris pH 7.4
100 mM tris / 1.5 M NaCl / 10 mM EDTA pH 7.4 (this is 10x assay buffer)
1% BSA (not fatty acid free) in assay buffer
Chill buffers and rotor

1. Thaw 1 tube of rat liver microsomes (0.5 ml / total protein=20 mg/ml) and dilute to 3.5 mg/ml with 100 mM tris / 100 mM KCl / 10 mM $MgCl_2$ /pH 7.4 so that the resulting microsome solution is 50 mM tris /50 mM KCl/ 5 mM $MgCl_2$ at 3.5 mg/ml total protein. For example, take 1.429 ml of 100 mM tris / 100 mM KCl / 10 mM $MgCl_2$ /pH 7.4 buffer and mix with 0.929 ml distilled water plus 0.5 ml microsomes.
2. Add 0.1 x volume of deoxycholate solution while vortexing and keep on ice. For example, add 3 x 95 µl in separate steps while vortexing and chilling.
3. Incubate for 30 minutes on ice.
4. Centrifuge at 105,000 x g for 75 minutes.
5. Recover supernatant and discard pellet.
6. Add 0.1 volumes of 10x assay buffer (100 mM tris / 1.5 M NaCl / 10 mM EDTA pH 7.4)
7. Use directly in the assay with CP-346086. The final protein concentration was 1.65 mg/ml.



Assay Validation

1. Dissolve CP-346086 in DMSO at the appropriate concentrations. For example, compound dilutions may be prepared at 5.5, 1.11, 0.55, 0.111 and 0.011 µM. Addition of 1 µl of each dilution to 100 µl of assay volume will give a range of inhibitor at: 55, 11.1, 5.5, 1.11 and 0.111 nM.
2. Pre-mix assay buffer / donor / acceptor and BSA to give 0.1% final concentration of BSA and use a final assay volume of 100 µl. For example, use 2 µl of donor, 2 µl of acceptor 10 µl of 1% BSA and 80 µl of assay buffer per assay.
3. Pipet the mix into the wells of a microplate.
4. Add 1µl of each dilution of CP-346086 to the wells and mix with a pipet.
5. Add 5 µl (8.25 µg protein) of purified MTP and incubate at 37°C for 60 minutes.
6. Read the plate at excitation 465 nm and 535 nm emission.

Assay Results

Raw FIU			CP-346086 (nM)
1363	1488	1392	55.000
1519	1517	1485	11.100
1632	1543	1639	5.500
1855	1877	1849	1.110
1887	1891	1858	0.55
2008	1978	1890	0.110
1878	1931	1952	0.000
1426	1571	1389	Blank

Technical Tips

- Donor and acceptor may be mixed with buffer and dispensed as one step.
- Results from duplicate samples should be tight. Variability indicates evaporation, inaccurate pipetting or incomplete mixing of assay components.
- Microplates containing samples may be incubated overnight at 25°C for more convenient reading schedules. MTP remains active at 25°C.
- Fluorescent assays are highly sensitive and will respond to slight changes in assay volume - BE SURE TO CAP TUBES.
- Microplates must be compatible with fluorescent assays. Some clear plates contain fluorescent plastic. We recommend black microplates (top-reading plate readers only) such as these: round bottom, black microplates (Corning, Costar #3792).
- The filter must be within specifications. An excitation filter of 485 nm with a 20 nm bandwidth may NOT be used. This filter will incompletely excite the label and the standard curve will appear to work, but protein activity results will be low.

Related Product

MTP, partially purified, human (Roar #RB-HLV), Unit size: 0.5 ml at 20 mg/ml.

For Research Use Only. Not for Diagnostic or Therapeutic Purposes.

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