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Product Information

MTP Activity Assay Kit
Supplied by Roar Biomedical, Inc

Catalog Number **MAK110**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

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

Components
The kit is sufficient for 100 assays in 200 µL total assay volume.

Donor Particle (concentration on label) Catalog Number MAK110A	0.4 mL
Acceptor Particle Catalog Number MAK110B	0.4 mL
MTP Assay Buffer Catalog Number MAK110C	20 mL

Reagents and Equipment Required but Not Provided.
- 96 well U-bottom black plates for fluorescence assays, with adhesive sealers
- 37 °C water bath incubator
- Fluorescence multiwell plate reader
- Spectrally pure Isopropanol (Catalog Number 34863)
- PMSF (Catalog Number P7626), for homogenization of HepG2 cells
- Leupeptin (Catalog Number L2884), for homogenization of HepG2 cells
- CP-346086 (Catalog Number PZ0103), for assay validation

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening.

Storage/Stability
The kit is shipped on wet ice. Storage at 2–8 °C, protected from light, is recommended. DO NOT FREEZE.

Components are stable for 1 year, if stored properly.

Procedures
All samples and standards should be run in duplicate.

Standards for Fluorometric Detection

1. Prepare six test tubes labeled from T0 to T5, each containing 1 mL isopropanol and add an additional 1 mL of isopropanol to T5.

2. Pipette 5 μL of Donor Particle mixture into test tube T5 and thoroughly mix (vortex) to adequately disperse the donor particles in the isopropanol.
   Note: The concentration of fluorescent substrate in the Donor Particle mixture is listed on the vial label.

3. Make four serial 2-fold dilutions (transfer 1 mL of previous standard solution to a tube with 1 mL of 2-propanol). For example transfer 1 mL of the mixture in tube T5 to tube T4 and vortex. Use tube T0 with isopropanol only as the 0 (Blank) Standard.
   Note: DO **NOT** incubate the standards.

4. Measure the fluorescence intensity (λ_{ex} = 465/ λ_{em} = 535) of the standards from tubes T0 to T5. For example, pipette 100 μL of each tube to a separate well of a plate and read the plate.

5. Create a standard curve by plotting the fluorescence intensity units of each standard versus the pmole amounts of fluorescent substrate in the Donor Particle mixture in each standard based on the concentration listed on the label.

Sample Preparation – Homogenization of HepG2 Cells

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

2. Prepare a sufficient amount of Homogenization Buffer (10 mM Tris, pH 7.4, with 150 mM NaCl and 1 mM EDTA).

3. To 100 mL of Homogenization Buffer, add 0.5 mL of 100 mM PMSF (Catalog Number P7626) in ethanol and 2 mL of 1 mg/mL leupeptin (Catalog Number L2884) in Homogenization Buffer.

4. Suspend the cells from 6 flasks in a total of 5 mL of homogenization buffer (protein concentration ~10 mg/ml).

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

6. Use 100 μg of protein homogenate in the MTP activity assay.
   Note: 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 Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. Add 190 μL of the Master Reaction Mix to each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
MTP Assay Buffer	182 μL
Donor Particle	4 μL
Acceptor Particle	4 μL

2. Add 10 μL of desired MTP source (homogenized HepG2 cells or partially purified MTP) to the appropriate wells. For a sample blank, add 10 μL of Assay buffer in place of MTP source.
   Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

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

3. Seal plate and incubate for 3–6 hours at 37 °C.
   Note: The microplate incubator must be able to rapidly raise the assay temperature to 37 °C. Large, humidified air incubators may cause problems by slowly increasing the temperature from 25 °C to only 34 °C after three hours. Floating the plate in a water bath is recommended, rather than using an air incubator.

4. Measure the increase in fluorescence of samples using a fluorometer (λ_{ex} = 465/λ_{em} = 535 nm). Determine the fluorescence intensity in the plasma or serum samples by subtracting the fluorescence intensity of the sample blank from each sample.

5. Calculate pmoles of label in the assay from the standard curve.

Assay Validation Procedure

Purified MTP must be used with CP-346086 (Catalog Number PZ0103). In this procedure, MTP was purified from rat liver microsomes.

Reagent Preparation

- Buffer A – 50 mL of 100 mM Tris, pH 7.4, with 100 mM KCl and 10 mM $MgCl_2$
- 50 mM Tris, pH 7.4, with 0.54% deoxycholate
- Buffer B – 100 mM Tris, pH 7.4, with 1.5 M NaCl and 10 mM EDTA
- 1% BSA (not fatty acid free)

Sample Preparation

Note: Chill buffers and rotor

1. Thaw rat liver microsomes (0.5 mL, total protein equal to 20 mg/mL) and dilute to 3.5 mg/mL with Buffer A so that the resulting microsome solution is 50 mM Tris, pH 7.4, with 50 mM KCl and 5 mM $MgCl_2$ at 3.5 mg/mL total protein. For example, take 1.429 mL of Buffer A and mix with 0.929 mL of water and 0.5 mL microsomes.

2. Add 0.1× volume of deoxycholate solution while vortexing and keep on ice. For example, add three separate 95 μL aliquots while vortexing and chilling.

3. Incubate on ice for 30 minutes.

4. Centrifuge at 105,000 × *g* for 75 minutes. Recover the supernatant while discarding the pellet.

5. Add 0.1× volumes of Buffer B. Use this directly in the validation assay with CP-346086.

Validation Reaction

1. Dissolve CP-346086 in DMSO at the appropriate concentrations (for example, 5.5, 1.11, 0.55, 0.111, and 0.011 μM).

2. Set up the Validation Master Reaction Mix according to the scheme in Table 2. Add 94 μL of the Validation Master Reaction Mix to each reaction (well).

Table 2.
Validation Master Reaction Mix

Reagent	**Volume**
MTP Assay Buffer	80 μL
Donor Particle	2 μL
Acceptor Particle	2 μL
1% BSA	10 μL

3. Add 1 μL of each CP-346086 dilution to the wells and mix by pipetting. Add 5 μL of the purified MTP, again mixing well by pipetting. Generating final inhibitor concentrations of 55, 11.1, 5.5, 1.11, and 0.111 nM.

4. To a Sample Blank, add 5 μL of MTP Assay Buffer and 1 μL of DMSO, in place of sample and CP-346086 solutions.

5. Seal plate and incubate for 60 minutes at 37 °C.

6. Measure the fluorescence of validation samples using a fluorometer (λ_{ex} = 465/λ_{em} = 535 nm).

Figure 1.
MTP Activity in Rat Liver Microsomes

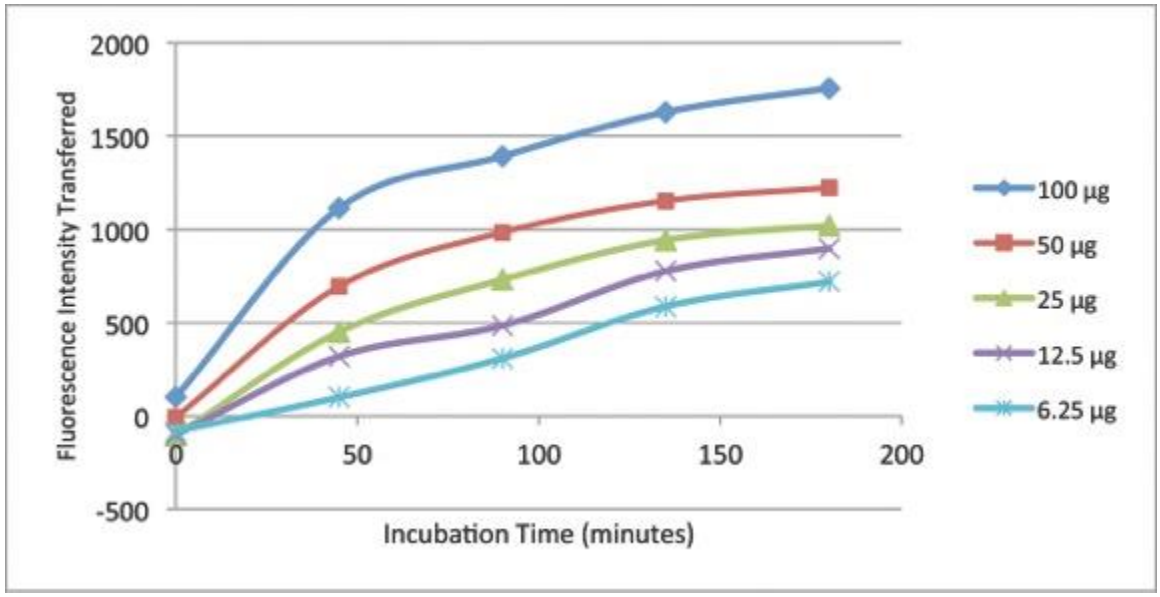
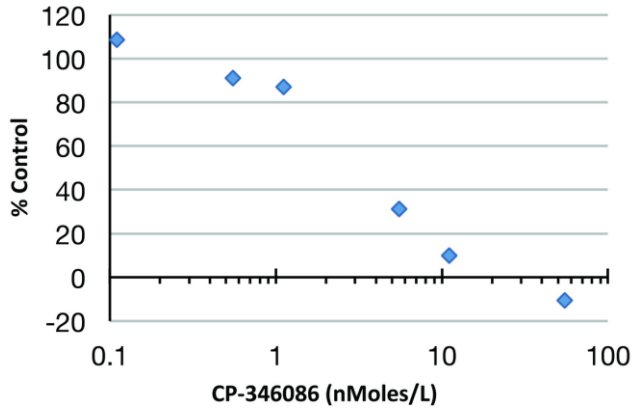


Figure 2.
MTP Inhibition with CP-346086



Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

This product is supplied by Roar Biomedical, Inc. and covered by several patents including U.S. Pat. Nos. 5,585,235; 5,618,683; 5,770,355, and related US and foreign patents.

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