Procedure for pancreatic lipase inhibitory activity assay (micelle method)

Written by Eisuke Kato on 2013/10/09
Modified on 2017/09/27
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Reagents

1. Porcine pancreatic lipase (Sigma, L3126)
2. Glyceryl trioleate (Sigma, T7140-1G)
3. L-α-Phosphatidylcholine (Sigma, P4279, purity>99%)
4. Taurocholic Acid Sodium Salt (Nacalai tesque, 32729-74)
5. Cetilistat (Lipase inhibitor, TCI, C2745)
6. NEFA C-testwako (FUJIFILM Wako Pure Chemical Co., 279-75401)
7. Several solvents.

Preparation of reagent solution

A. Tris-HCl buffer
   Prepare 13 mM Tris-HCl buffer, pH 8.0, with 150 mM NaCl and 3 mM $CaCl_2$)
B. Porcine pancreatic lipase solution
   Dissolve 4.5 mg of Porcine pancreatic lipase in 30 mL of Tris-HCl buffer.
   ***Prepare fresh solution before the assay**
C. Glyceryl trioleate solution
   Dissolve 1.0 g of Glyceryl trioleate in 6.25 mL of chloroform. Store under nitrogen atmosphere at -20 ºC.
D. L-α-Phosphatidylcholine solution
   Prepare 100 mg/mL ethanol solution. Store at -20 ºC.
E. Stop reagent
   Prepare 1 M aq. HCl.
F. Positive control (cetilistat)
   Prepare 64 μM solution in DMSO. Store at -20 ºC. Dilute 2 times with water to prepare 32 μM/50% aq. DMSO solution before the assay.

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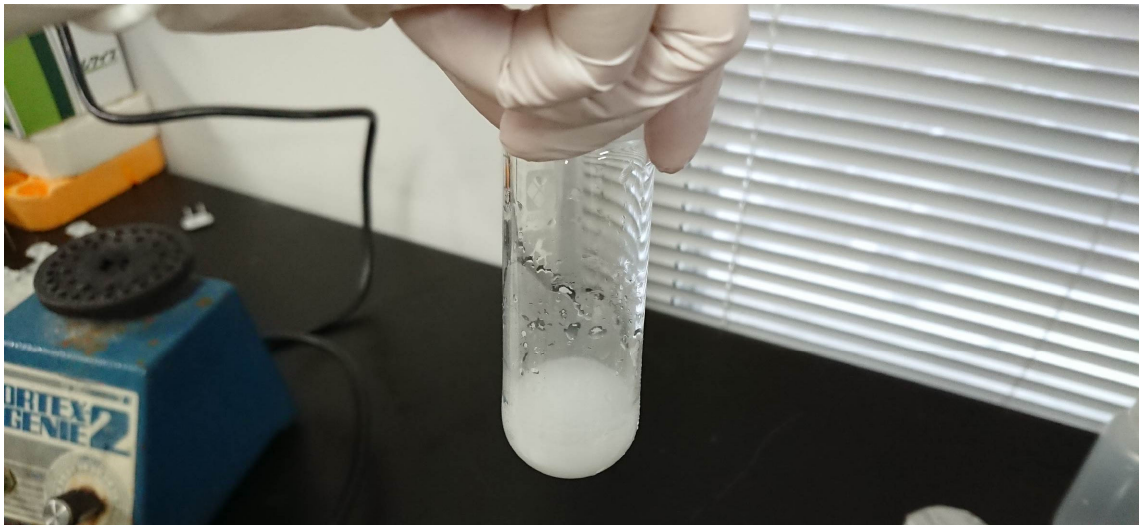
Preparation of glyceryl trioleate emulsion

1. Add 200 μL of Glyceryl trioleate solution and 200 μL of L-α-Phosphatidylcholine solution in a glass tube and evaporate the solvent.
2. Add taurocholic Acid Sodium Salt (10 mg) and **cold*** Tris-HCl buffer (9 mL) in the tube.
   *Ice cooled or stored in refrigerator.
3. Vortex the tube for 20 seconds and make a suspension
4. On ice, sonicate the suspension using ultrasonic homogenizer for 5 min and create a micelle solution (duty cycle: constant, output control: 3, time: 5 min, with Branson Sonifier 250)
   *The prepared emulsion with be like bellow photo.



Assay procedure

1. Add 200 μL micelle solution and 100 μL sample solution (in 50% DMSO aq.) to 1.5 mL micro tube, mix and pre-incubate for 5 min at 37 ºC*
2. Add 100 μL of lipase solution, mix and start the reaction
3. After reacting for 30 min at 37 ºC, add 40 μL of stop reagent. Mix well.
4. Add 600 μL of hexane
5. Mix rigorously for 60 sec. and leave settled until phase separation
6. Repeat step 5 for three times
7. Take 300 μL from hexane layer to the 0.6 mL micro tube
8. Evaporate the hexane and dissolve the residue in 100 μL of DMSO.
9. Measure the oleic acid in the DMSO solution by NEFA C-testwako kit.

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Required mixtures

	Micelle sol.	Sample sol.	Lipase	Buffer
Control	200 μL	100 μL	100 μL	
Control Blank	200 μL	100 μL		100 μL
Sample	200 μL	100 μL	100 μL	
Sample Blank	200 μL	100 μL		100 μL

Calculation

$$\text{Inhibitory activity (\%)} = \left(1 - \frac{A_{550}\text{sample} - A_{550}\text{sample blank}}{A_{550}\text{control} - A_{550}\text{control blank}}\right) \times 100$$