Glucose Uptake Assay

Glucose uptake [Kozma, 1993 #876] – 3T3-L1 Cells (5 X 10^5/well) plated in a 12 well plate in serum-containing medium (DMEM) overnight were washed twice in PBS, and incubated in serum-free medium for 2 hr, then incubated in 1 ml/well PBS containing 200 nM insulin for 30 minutes at 37 ^oC. After one wash in PBS, the cells were incubated in 1 ml PBS containing 0.1 mM 2-deoxyglucose and 1 μCi/ml 2-deoxy-D-[^{3}H] glucose for 5 min, then, washed three times in ice-cold PBS, solubilized in 0.4 ml of 1% SDS. ^{3}H-glucose uptake was detected in 4 ml of scintillant using Beckman LS6500 scintillation counter. Nonspecific deoxyglucose uptake is measured in the presence of 20 μM cytochalasin B and is subtracted from the total uptake to get specific glucose uptake.

1. 3T3-L1 cells 2 x 10^5/ml/well in 12 well plate overnight

2. Wash 3 X in PBS

3. RPMI-1640 with 1% BSA 1 ml/well for 2 hrs

4. Add calyculin for 20 mins

5. Wash in warm PBS 2 X

6. Add 1 ml PBS/well that containing 200 nM insulin [add 1 ul of insulin stock (10 mg/ml =1.73 mM) to 9 ml of PBS].

7. Incubate for 30 min at 37 degree

8. Wash 2 X in warm PBS

9. Add cytochalasin B to negative control at 20 uM. Add 1ml/well warm PBS containing 0.1 mM 2-dG and 1 uCi H3-2-DG to every well.

   2-dG stock (D-8375, Sigma, 1 g , in 60 ml PBS): 100 mM at 4 degree. 1:1000 dilution to get 0.1 mM.

   H3-dG (TRK672, Amersham): 1 uCi/ul.

   Cytochalasin B (C-6762, Sigma, 1 mg in 100 DMSO): 20 mM. 1:1000 dilution.

10. Wash 3 X in cold PBS

11. 0.4 ml 1% SDS/well, Rt for 10 min to lyze the cells

12. Transfer the whole cell lysate completely into 4 ml scintillant and counting CPM