



(Figure 2.8): The diluted cells were plated at a desired volume on cell culture plates (96-wells).

2.3.9.5. Cell-based insulin degradation assay

Normal and diabetic rat hepatocytes were grown to subconfluence on a 69-well dish. One well should be a cell-free control in each study. The appropriate amount of human insulin (100 nM, 200 nM) was applied to the cell cultures. 100 μ l of medium was collected after 15, 30 and 60 min of incubation and the insulin concentration was measured by Elecsys 2010 analyzer.

2.3.9.6. The inhibitory effect of bacitracin on cell-mediated insulin degradation in isolated hepatocytes

Normal and diabetic rat hepatic cells were grown to subconfluence on a 69-well plate. One well should be a cell-free control in each study. Each of concentrations of bacitracin (300 μ M, 1 mM) was applied for plate of 100 nM human insulin. Similarly, each of the same amounts of bacitracin was applied on 200 nM insulin. 100 μ l of