Extracellular ATP detection of primary cultured microglia

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Abstract

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Before start

When open vials and bottles, you should make sure that the contents of these are not contaminated with ATP.

Materials

ATPlite one step kit by Perkin Elmer

Protocol

Step 1.

Lyophilized substrate solution was reconstituted using the appropriate volume of buffer.

Step 2.

A vial of lyophilized ATP standard solution was reconstituted with water.

Step 3.

ATP standard solution was diluted as follows: 1000pM, 500pM, 250pM, 125pM, 62.5pM, 31.2pM, 15.6pM, 7.8pM.

Step 4.

For detection, we used 384-well opaque plates, with each well containing either 25 µl sample or ATP standard.

Step 5.

Another 25 µl substrate solution was added to each well.

Step 6.

The 384-well microplate was shaked for 2 minutes at 1,100 rpm.

Step 7.

A multifunctional microplate reader (PerkinElmer, USA) was used to measure the luminescence of each group.

Step 8.

The ATP content was calculated according to a standard curve drawn by CurveExpert V1.4.

Step 9.