

Applications

Note 209 | May 2009

Apoptosis assay with the Promega Apo-ONE® Homogeneous Caspase-3/7 Assay on the epMotion® 5075 TMX

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Abstract

Apoptosis is an important cellular mechanism which plays a role in many diseases. Now, an automated system for the performance of an apoptosis assay is available via the combination of the Apo-ONE® Homogeneous Caspase-3/7 Assay by Promega and the epMotion® 5075 TMX.

Introduction

Apoptosis is a cellular program which enables superfluous or damaged cells to eliminate themselves almost without adverse effects on surrounding cells. This “programmed cell death” is a natural occurrence during development, as well as in the adult organism, in order to give organs their shape, or during the elimination of superfluous or potentially harmful cells of the immune system. However, numerous findings point towards an involvement of apoptosis, or part of the apoptosis program, in many acute or chronic neurodegenerative diseases such as Parkinson’s, Huntington’s and Alzheimer’s disease. Insufficient apoptosis can be found, for example, in cancer and autoimmune diseases. Hence, apoptosis and its effects are important factors in the investigation of diseases [1, 2, 3].

The process of apoptosis can be divided into two phases: the initiation phase and the sentencing/execution phase. The initiation phase can be triggered by intrinsic signals, i.e. the cell itself recognizes that it is superfluous or harmful, or by extrinsic signals, meaning that the cell receives external apoptotic signals. During apoptosis, caspases, enzymes with proteolytic activity, play a crucial role. During the initiation phase, the caspase cascade is triggered, which will activate the execution caspases, mainly caspases 3, 6 and 7 [1, 2, 3]. The activity of caspases 3 and 7 is measured using the Promega Apo-ONE Homogeneous Caspase-3/7 Assay [4]. The automation of this assay eliminates errors due to personnel-specific pipetting inaccuracies, thus making available a standard protocol for reproducible performance of this assay.

Materials and Methods

epMotion 5075 TMX

Single channel dispensing tool for 40-1000 μ l
Reservoir rack
Reservoir rack module for Safe-Lock tubes
Reservoir rack module for 15 ml tubes
epT.I.P.S Motion, 1000 μ l
Safe-Lock reaction tubes 1.5 ml

Reagents and accessories by other manufacturers:

NIH-3T3 (mouse fibroblasts)
DMEM
10 % FBS superior
Trypsin 0.25 % with EDTA
D-PBS
Apo-ONE Homogeneous Caspase-3/7 Assay (Promega, G7790)
Staurosporine in DMSO
 μ Clear Plate 96, black TC (Greiner)
15 ml tubes
Plate reader: Safire II™ (Tecan)

The following day, the cells were treated with two different concentrations of staurosporine (1 μ M and 10 μ M), an apoptosis-triggering reagent. The creation of the staurosporine dilutions, as well as their distribution into the wells, was performed using the epMotion 5075 TMX [5]. To this end the staurosporine was introduced, and the respective concentrations in medium were prepared in the two 1.5 ml Safe-Lock tubes. Subsequently, these dilutions were distributed onto the plate using Reagent Transfer [5]. The cells were then incubated for 5 h at 37 °C in a 5 % CO₂ atmosphere and 100 % humidity. Following this incubation, the Apo-ONE Homogeneous Caspase-3/7 Assay was performed using the epMotion 5075 TMX [5]. To this end, the buffer was transferred to a 15 ml tube. Then the buffer and substrate from the assay kit, along with the sample plate, were placed onto the worktable [5]. The caspase reagent was mixed together from the substrate and buffer. Subsequently, 100 μ l of caspase reagent were distributed into each well of the plate, using Reagent Transfer [5], followed by 30 minutes of assay incubation on the Thermomixer of the epMotion 5075 TMX at 37 °C and slight shaking at 350 rpm. At this point, the assay could be measured at the wavelengths 499/521 nm in the Safire II plate reader. The medium with caspase reagent was used as the blank value, and cells incubated without staurosporine, but with caspase reagent, were used as a negative control.

Procedure

NIH-3T3 cells cultivated in DMEM were seeded onto a black 96 well microtiter plate with clear bottom at a density of 33,000 cells/well. In order to ensure adhesion, the cells were incubated over night at 37 °C in a 5 % CO₂ atmosphere and 100 % humidity.

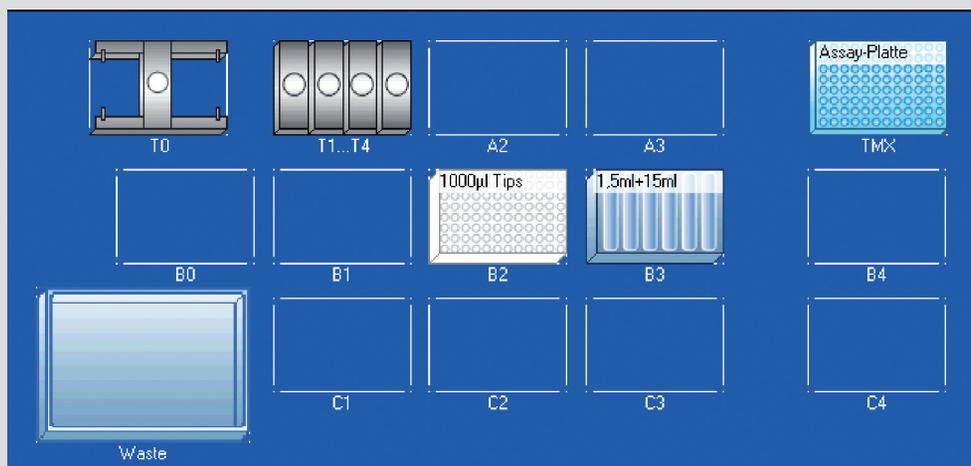


Figure 1: Worktable layout on the epMotion 5075 TMX for the performance of the Apo-ONE Homogeneous Caspase-3/7 Assay. The identical layout was used for the creation of the staurosporine dilutions.

Results

Figure 2 shows that apoptosis of the NIH-3T3 cells intensifies with increasing concentrations of staurosporine. The fluorescence of the medium alone (= blank) was subtracted from all other values. The average blank value was 853 RFU. As expected, the autofluorescence of the cells in medium lay above this value. The maximum concentration of 10 μM staurosporine was used to ensure cell death. The concentration of 1 μM was chosen as a comparative value.

Conclusions

The Apo-ONE Homogeneous Caspase-3/7 Assay is ideally suited for automation on the epMotion 5075 TMX. The integrated Thermomixer allows assay incubation without further manual handling, thereby ensuring reproducible incubation conditions. In addition, the easy but flexible programming and use of the epMotion 5075 TMX, equipped with the user software epBlue, allows for reproducible distribution of the appropriate dilutions of the cytotoxic substances.

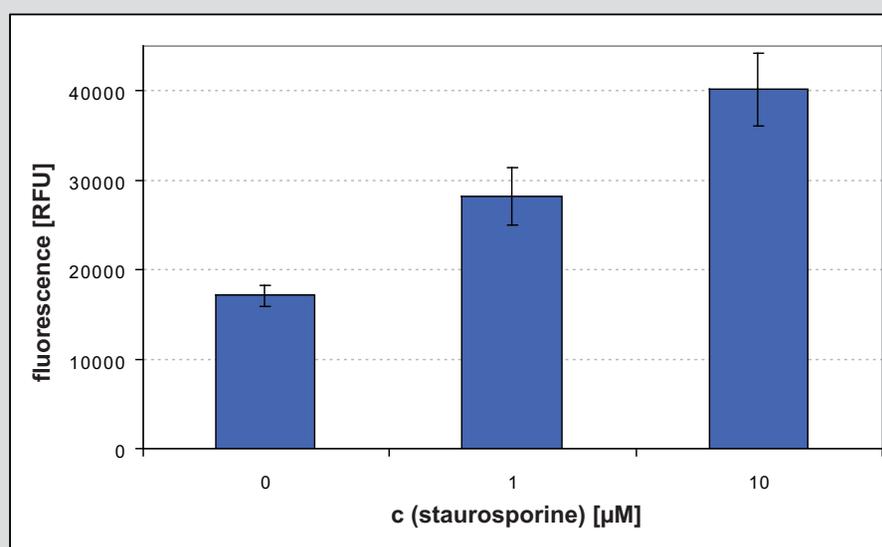


Figure 2 : Staurosporine-induced apoptosis of NIH-3T3cells

References

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- [2] Stefan Grimm: Apoptosis: Programmed cell death (Die Apoptose: Programmierter Zelltod [German]); *Chemie in unserer Zeit* 37(3), 2003, pp. 172–178
- [3] Fritz Höffeler: The apoptosis machinery: Chronicle of a death foretold (Die Maschinerie der Apoptose: Chronik eines angekündigten Todes [German]); *Biologie in unserer Zeit* 34(1), 2004, p. 1623
- [4] Promega, *Technical Bulletin* Apo-ONE Homogeneous Caspase-3/7 Assay
- [5] Eppendorf user manual for epMotion 5075

Ordering Information Eppendorf

Product	Order no. international	Order no. North America
epMotion® 5075 TMX	5075 000.784	960020404
Single-channel dispensing tool TS 1000	5280 000.053	960001036
Reservoir Rack	5075 754.002	960002148
Thermorack for 96 PCR Plates	5075 766.000	960002083
Reservoir Rack Module for Safe-Lock	5075 799.081	960002620
Reservoir Rack Module for 15 ml reaction tubes “Blue Cap”	5075 799.162	960002650
Safe-Lock tubes 1.5 ml	0030 120.086	022363204



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