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Datasheet MEF cells

Introduction

Mouse embryonic fibroblasts (MEF) are used as feeder cells to support growth of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) by excreting necessary nutrients, some of which are still unidentified. This primary murine cell line is often stored in liquid nitrogen and cultured 24 hours before a feeder layer is required for the support of ESCs or iPSCs. Whether these cells could be stored at 4°C to fully utilize cells cultured from liquid nitrogen and to determine if transportation of cells at 4°C is a possibility, Rokepie-S01® was tested on MEF cells in early passage.

Storage of cells

$2{,}5^{E}5$ cells/mL (5 mL in total) were cultured in T-25 flasks with standard phenolic-style screw caps (not vented) in DMEM without pyruvate (Invitrogen, cat. No 41965), supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 µg/mL streptomycin. After 24 hours of culturing in a humidified incubator, Rokepie-S01® was added to the cell culture medium and cells were incubated for two hours. Subsequently, caps were closed air-tight and the cells were transferred to a cold room for storage (4°C ±1°C). After 7 and 14 days, flask were removed from the cold room and placed in a humidified incubator with loosely opened caps to allow gas exchange. 24 hours after removing the cells from cold storage, general morphology and cell attachment was assessed, after which viability was determined by Trypan blue exclusion.

Results

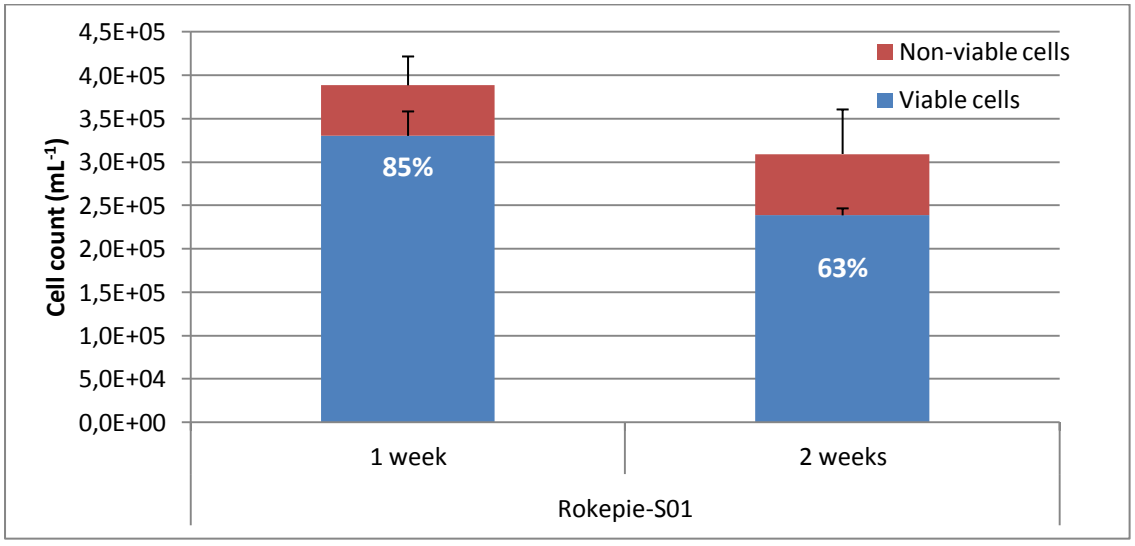

Figure 1 Cell number and viability of MEF cells after 1 (left) and 2 (right) weeks of storage at 4°C. Viability was determined 24 hours after cold storage by Trypan blue exclusion, data is shown as mean +SD (n=3).

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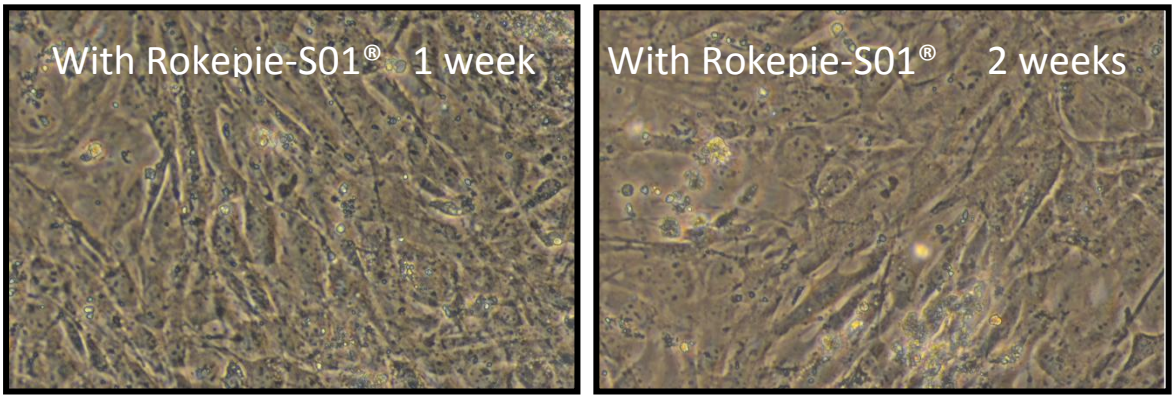

Figure 2 MEF cells in medium supplemented with Rokepie-S01® after 1 week of storage (left) and 2 weeks of storage (right) at 4°C (25x magnification).

Summary

The addition of Rokepie-S01® allowed for the successful cold storage of MEF cells for up to two weeks. When compared to the amount of cells seeded prior to cooling, almost 100% of the starting amount of cells (=2,5^{E}5) showed viability, according to the Trypan blue exclusion method. Very little cell detachment was observed during and after cooling. Whereas other cell lines curl up or even detach during cooling (without a loss of viability), MEF cells stayed attached during the entire storage period. Possible applications of cold storage of MEF cells is for the end user to decide, as well as the functionality of these cells in a feeder layer supporting iPSCs and ESC.