ROKEPIE®

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Datasheet MS5 cells

Introduction

The murine MS5 bone marrow stromal cell line can be used to support growth of induced pluripotent stem cells (iPSCs) and hemotopoietic stem cells (HSCs). These cells are often thawed and cultured 24 hours before a batch of stem cells is ready for culturing and support of growth is required, something that can occur several times a week. Normally, no more than 2-3 wells on a 6-well plate are required and the protocol that is used to thaw these cells and get rid of the toxic cryoprotectant can take up to an hour, making this a labor intensive necessity. If it could be possible to seed a week's worth of cells on a single day, possibly supplying the entire department with ready plated 6-wells plates from a single batch, this could save researchers and technicians several hours per week.

Culturing and storage of cells

$2{,}0^{E}5$ cells/mL MS5 were cultured in a T-25 flask with standard phenolic-style screw caps (not vented) in DMEM (Invitrogen, cat. No 41965), supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 µg/mL streptomycin and a non-confluent monolayer was obtained 24 hours after seeding. Rokepie-S01® was added to the cell culture medium (1:10 and 1:100) and the cells were incubated for 2 hours in a humidified incubator (37°C, 5% CO_2). Subsequently, the caps were shut airtight to limit gas exchange and flasks were transferred to a cold room for storage. After 7 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.

Since 6-wells plates are the seeding vessel of choice for feeder layers, Rokepie-S01® was subsequently tested in this format. $4{,}0^{E}5$ cells/well were seeded 24 hours prior to cold storage as described above, and plates were stored in an airtight Ziploc bag. After 5 days of cold storage, the plates were removed from their plastic bags and placed in a humidified incubator for 24 hours before determining viability and morphology.

Results

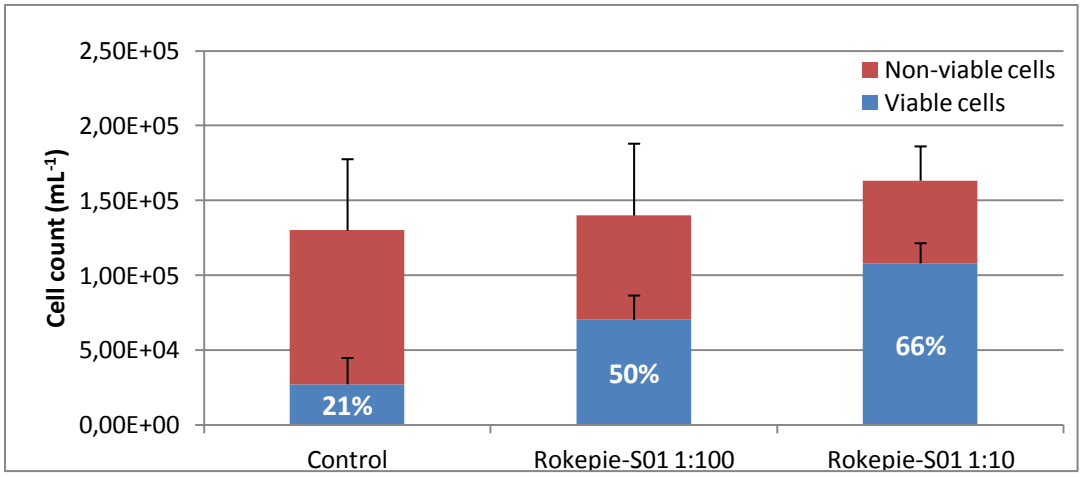

Figure 1 MS5 viability after 1 week storage in T-25 flasks at 4°C was determined by Trypan blue exclusion, expressed as mean +SD (n=3).

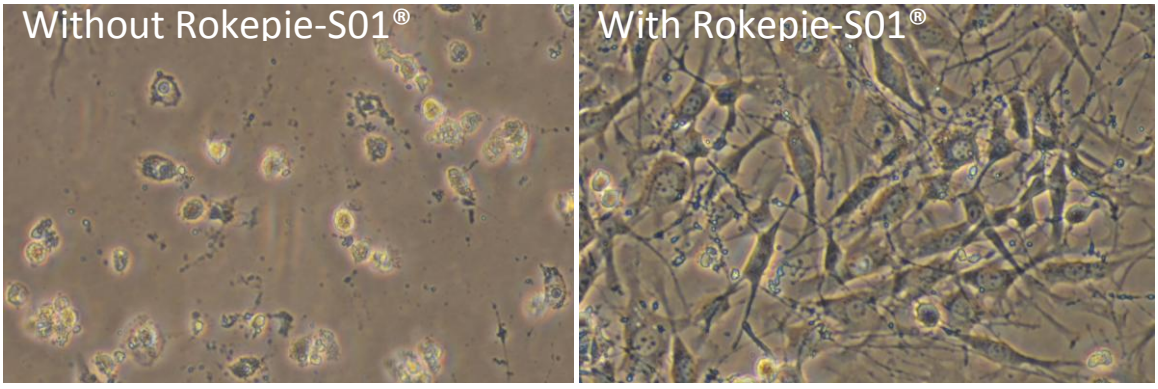

Figure 2 MS5 cells in standard cell culture medium (left) and medium supplemented with Rokepie-S01® (right) after 1 week storage at 4°C (25x magnification).

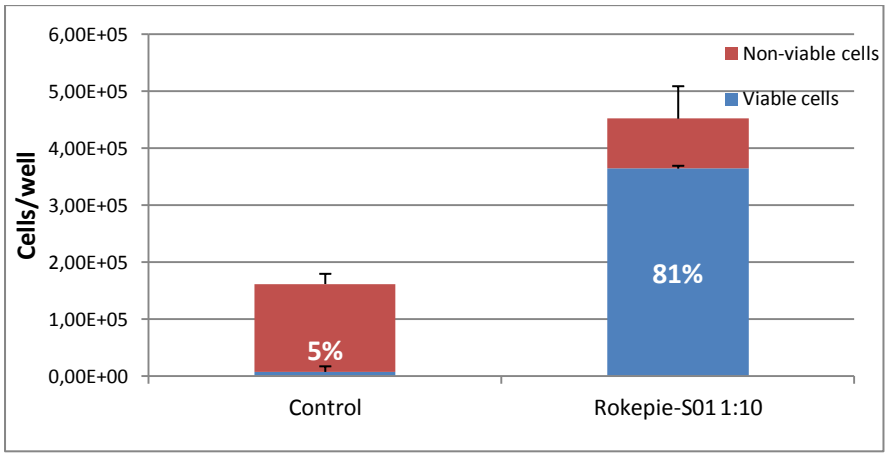

Figure 3 MS5 viability after 5 days of storage at 4°C in a 6-well plate was determined by Trypan blue exclusion and is shown as mean +SD (n=3).

Summary

Storage of MS5 cells was most successful after the addition of Rokepie-S01® in a 1:10 ratio to standard cell culture medium. Compared to control, a 40% increase in viability and minimal detachment of cells was observed in T-25 flasks and a 76% increase in 6-well plates. Since all required growth-supporting ingredients remain present during and after storage, cell growth is supported upon placing the cells back at 37°C. Although feeder layer cells are irradiated before use to limit proliferation, these experiments were performed with non-irradiated MS5 cells in early passage numbers (2-4). Whether irradiation should happened before or after storage, and if it affects post 4°C-storage viability should be determined by the end user. Whether MS5 cells still exhibit feeder layer properties after cold storage is under investigation.