Admiraal de Ruyterlaan 5, 9726 GN, Groningen, the Netherlands

Datasheet
Storage of cell suspensions at 4°C

Introduction

As an alternative to the storage of cells in a monolayer, the possibility to store cell suspensions of freshly trypsinized cells at 4°C was investigated. Storing cells at 4°C in suspensions could allow for technicians and other laboratory personnel to transport or receive suspended cells which are ready to be plated, eliminating the need of removal of toxic cryprotectants or carefully resuspending frozen cells to prevent a temperature- or osmotic shock. When cells are in suspension, it is easy to take a sample in order to determine viability and proceed with seeding. Additionaly, the cells can be cultured in any vessel: Once the density is known, one vial of cells can be divided over several wells or all transferred to one flask in order to propagate. Preparing the cells for storage at 4°C does not require regulation of cooling rate, as is the case with cryopreservation (storage in liquid nitrogen). Storage of cell suspensions at 4°C could also apply to cells that do now show adherent growth and could be especially usefull for cell lines vulnerable to cryopreservation or primary cells.

Culturing and storage of cells

In order to determine if Rokepie supports the 4°C storage of freshly trypsinized cells, the following experiment was performed: HEK293 cells were seeded in 6-wells plates on day t=-1. On day t=0, 6 wells recieved 1:10 Rokepie-S01 (added 160 µL to 1,5 mL. Group A&B). Cell culture medium was not replaced at this stage. After 2 hours of pre-incubation in Rokepie-S01, cells were washed twice with 1xPBS and trypsinized (250 µL), after which cells were resuspended in 3 mL of fresh cell culture medium per well. 6 wells recieved standard cell culture medium to which Rokepie-S01 was added in a 1:10 ratio (2 mL to 18 mL. Group A&C). See table below for a layout of the groups. Cells from each well were distributed among 2 1,5 mL eppendorf cups, which were immediately transfered to a cold room. The averag cell density prior to cold-storage was 4,7E5 cells/mL.

Group	Rokepie-S01 pre-incubation	Rokepie-S01 present during storage
A	+	+
B	+	-
C	-	+
D	-	-

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Results

In order to determine if cell attachment would occur after trypsinization and storage at 4°C, the remainder pooled in 5 mL of fresh cell culture medium. This cell suspension was then transferred to a T-25 flask, which was closed airtight and transferred to a cold room. 1 day after commencing storage at 4°C, no cell attachment was observed in this flask. After 2 days of cold storage, cells that were transfered to a 6-wells plate and placed in a humidified incubator showed attachment and changes in morphology after a few hours (data not shown).

After 1 and 2 days of storage at 4°C, samples were taken asceptically and viability was determined by Trypan blue exclusion. The cell suspension in 1,5 mL cups appeared looseley palleted due to gravtiy and could be resuspended easily by inverting the cups (data not shown). After 1 day of storage at 4°C, viability (%) was highest in groups A and B, which consists of cells pre-incubated with Rokepie-S01. However, this difference was not present after 2 days of storage at 4°C, since the total number of viable cells (as indicated by the blue bar) did not differ between groups A, B and C (figure 2).

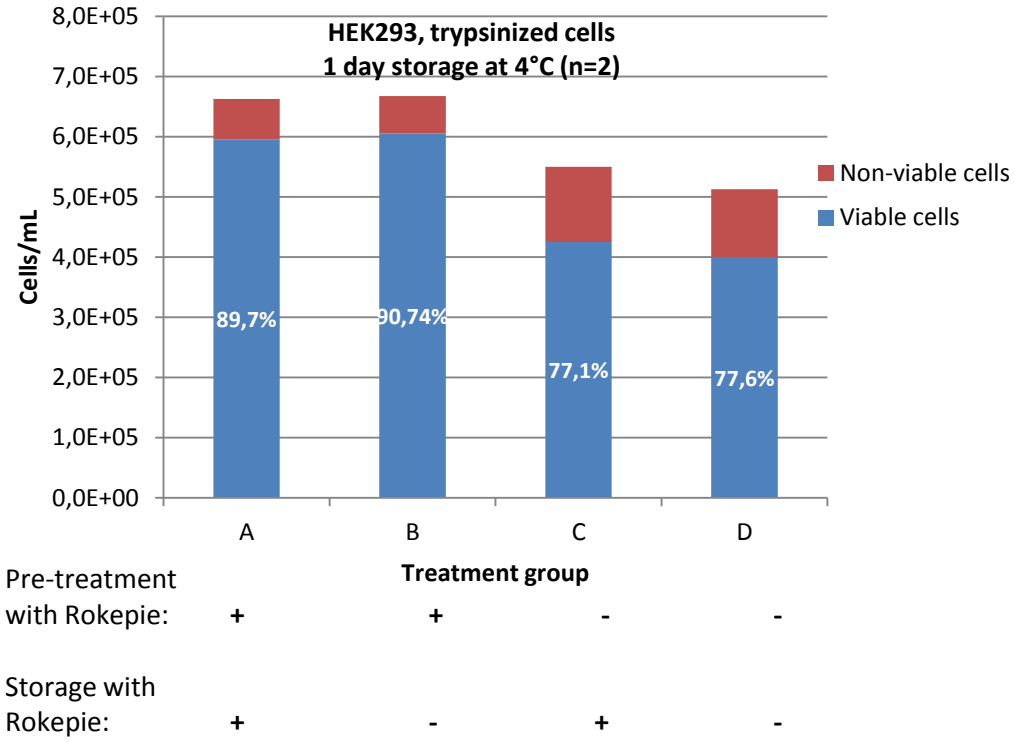

Figure 1 Viability of HEK293 cell suspensions stored at 4°C for 1 day was determined by Trypan blue exclusion. Data is shown as the concentration of viable cells (blue bar) and non-viable Trypan blue positive cells (red bar). The percentage of viability is shown in the bars. All data is presented as means, n=2.

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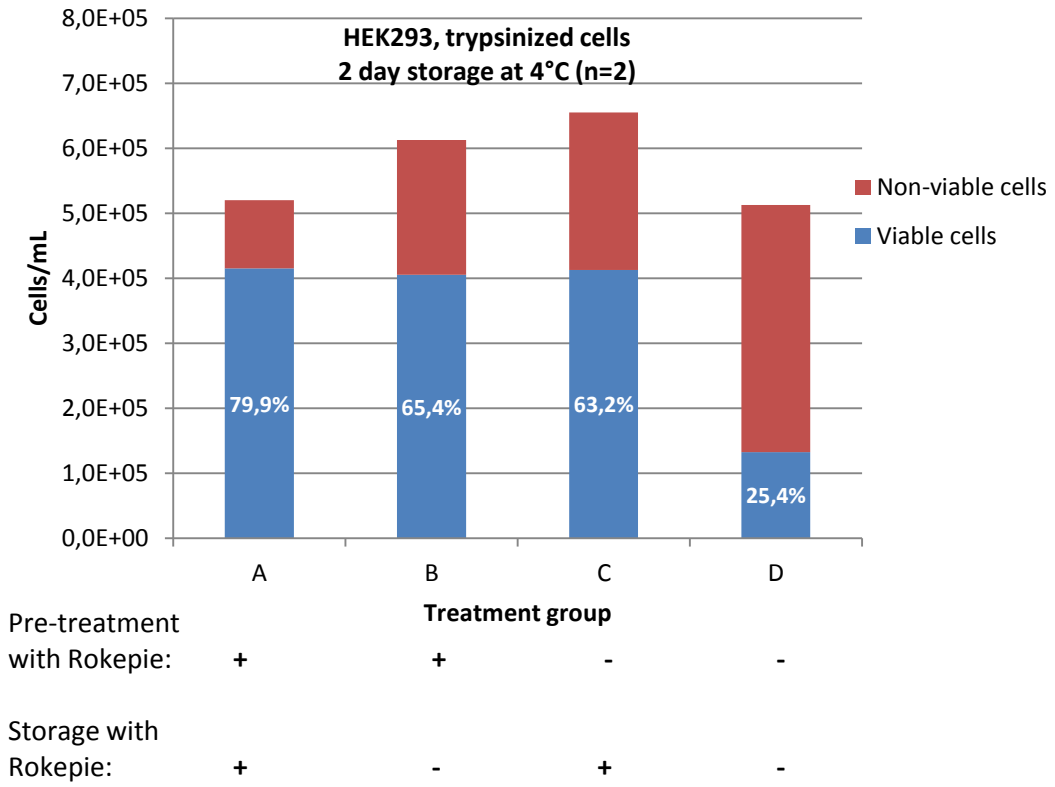

Figure 2 Viability of HEK293 cell suspensions stored at 4°C for 2 days was determined by Trypan blue exclusion. Data is shown as the concentration of viable cells (blue bar) and non-viable Trypan blue positive cells (red bar). The percentage of viability is shown in the bars. All data is presented as means, n=2.

Summary

Pre-incubation with- or addition of Rokepie-S01 to the standard cell culture medium allowed for storage of cells at 4°C for up to at least 2 days. Even though viability percentages differ, the total number of viable cells per millilitre are equal. It appears more cells are counted after storage at 4°C than the number of cells that entered storage (4,7E5 cells/mL on average). Whether this is due to cell doubling during cold storage, or due to a counting error of the start of the experiment is not certain.Future experiments will include variables that help determine the quality of the cells, such as glucose utilization, lactate concentration an pH. Following these future experiments, the cells will be used to set up a growth curve in order to determine of this storage method infleunces the growth rate in any way.