Storage of mammalian cells at 4 °C *with Rokepie-S01®*

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Introduction

A living, working model of hypothermic storage of mammalian cells exists in the form of hibernators. Research in the field of hibernation has led to the development of several new chemical entities which serve as bioregulators: These compounds help cells enter a hibernation-like in which the cells are protected against damage resulting from oxidative stress, which occures during cooling and rewarming. Hibernation is marked by changes in metabolism towards a *vita minima:* A minimal need for oxygen and nutrtition that allows for the storage of living cells at 4 °C for up to at least 7 days.

Target

Using the most advanced one of the aforementioned new chemical entities, a product was developed that helps spread the load of routine cell culture activities by allowing cells to be stored at 4°C. The product needed to be easy in use, straightforward and its application possible in cell culture laboratories, without the need of specialized cell culture media or equipment.

This led to the development of **Rokepie-S01®**, an isotinic, sterile solution which is ready for use in cell culture upon arrival. **Rokiepie-S01®** is free of animal-products, antibiotics and preservatives and does not contain solvents like ethanol or DMSO.

Materials & Methods

Flask model: Several cell lines are seeded in T-25 flasks at fixed densities. 24 hours after seeding, **Rokepie-S01®** was added to the cell culture medium (*i.e.* cell specific, generally DMEM, 4,5 g/L glucose, no pyruvate, 10% FCS and antibiotics) and cells were incubated for 2 hours (37 °C, 5% CO_2, humidified). Subsequently, caps were closed airtight and flasks are stored in a cold-room for several days to weeks. Viability was assessed by Trypan Blue exclusion 24 hours after removing cells from cold storage and placing them in a humidified incubator with opened caps. A growth curve was established by seeding cells in 6-wells plates following cold storage and determining viability daily. Cytotoxicity was measured according to ISO 10993-5:2009. HEK293 cells were plated 24 prior to the cytotoxicity assay and recieved several **Rokepie-S01®** dilutions in fresh culture medium. Cells were exposed to **Rokepie-S01®** for 24 hours at 37°C, after which Neutral Red absorption by viable cells was determined. Control cells recieved fresh culture medium only. The highest dose of **Rokepie-S01®** was 5x the advised working concentration.

Results

Flask model: **Rokepie-S01®** was tested on the following cell lines. Storage was deemed succesfull whenever viability ≥ 65% was measured.

Cell line	Application (e.g.)	4 °C Storage duration (at least)
CaCo2 (carcinoma)	*In-vitro* drug absorption	7 days
HEK293 (immortalized)	Transfection	7 days
MEF (primary, embryonic)	Feeder-layer for iPSCs	7 days
MS5 (primary, embryonic)	Feeder-layer for iPSCs	7 days
Skov-3 (carcinoma)	Transfection	2 days
Platelets/thrombocytes	Transfusion	21 days

Growth after 7-day storage at 4°C of HEK293 cells was compared to 37°C control cells. Viability and cell number was determined by Trypan Blue exclusion and was expressed as viable cells /cm^2.

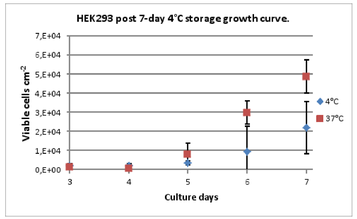

Figure 1. Growth curve of HEK293 following a 7-day period of storage at 4°C. Cells were seeded after determining cell number and viability and recieved fresh cell culture medium every 2-3 days. Equal numbers of viable cells were seeded. Cell number and viability were assessed daily. Data is expressed as viable cells/cm2 +/- SD (n=3).

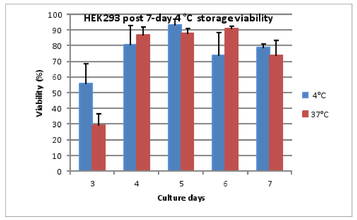

Figure 2. Viability was assessed daily by Trypan Blue exclusion. Data is expressed as an average +SD (n=3)

Rokepie-S01® cytotoxicity was determined according to ISO regulations on HEK293 cells.

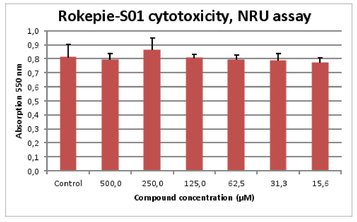

Figure 3. NRU viability assay after 24 hours of exposure of HEK293 cells to Rokepie-S01® in standard cell culture medium. Data is expressed as an average +SD (n=6). Compared to control cells, Rokepie-S01® does not cause any decrease in the number of viable cells and shows no cytotoxicity.

Conclusions

Rokepie-S01® enables the storage of cells at 4°C for several days to weeks, depending on the cell line of choice. Functionallity is cell line-specific and needs to be determined by the end-user (*e.g.* HEK293 needs to be transfected, CaCo2 should show good permeability characteristics etc.). Hypothermic storage of cells could offers lab-technicians a new way of spreading or delaying workload without losing large numbers of cells, as would be the case with storage in liquid nitrogen. **Rokepie-S01®** can be of special interest in departments that use primary, vulnerable or special cell lines: Hypothermic storage allows for a quick start-up after storage, since a large amount (or surface) of cells can be stored and the standard cell culture medium supports growth immediately after cooling.

Continuous passage (37°C)	Cryostorage (-180°C)	Hypothermic storage (4°C)
+ Large batches: Cells always ready to go	+ Once storage is achieved, little attention is needed	+ Storage facilities readily available in labs
+ Standard, well defined medium is used	+ Cells can be stored for years	+ Pause. Confluent Friday ≈ confluent Monday
		+ Large batches: Short start-up after storage
– Requires continuous stream of recourses, space, attention (*i.e.* money)	– Exposure to (toxic) cryoprotectants can alter cell behavior/experiment outcome	– Cells need special storage solutions. Current solutions are proprietary formulation...
– "Zoo": Multiple cell-lines in culture, risk of cross-contamination increases	– Small batches: expanding 1 vial to experimental/production quantities can take several weeks	...thus exposing cells to uknown storage solutions. **But not with Rokepie-S01®, which is added to your well formulated medium.**
– No pause. Confluent today = overgrown tomorrow	– Large LN2 vessels require constant N_2 delivery