ROKEPIE®

CELL PROTECTION BY HIBERNATION

Protect and preserve cells and tissues at low temperatures

ROKEPIE®

sulfateq

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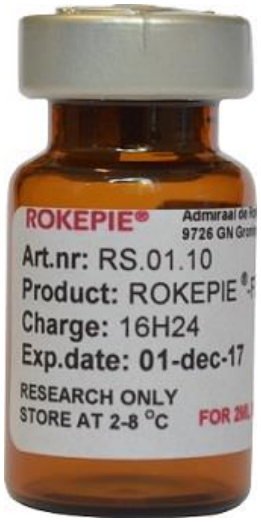
Product info

ROKEPIE® = a bioregulator to preserve cells & tissue at 2-8° C.



- Additive
  - No need to change medium
- Non-toxic
  - No need to remove before rewarming (37°C
  - Supports cell growth after rewarming
- RUO product
  - For Research Use Only
- Fully synthetic
  - No animal components, antibiotics and preservatives
  - One defined, small molecule: SUL-109
- Sterile additive
  - Open, Add, Incubate & Use
- Non-GMP

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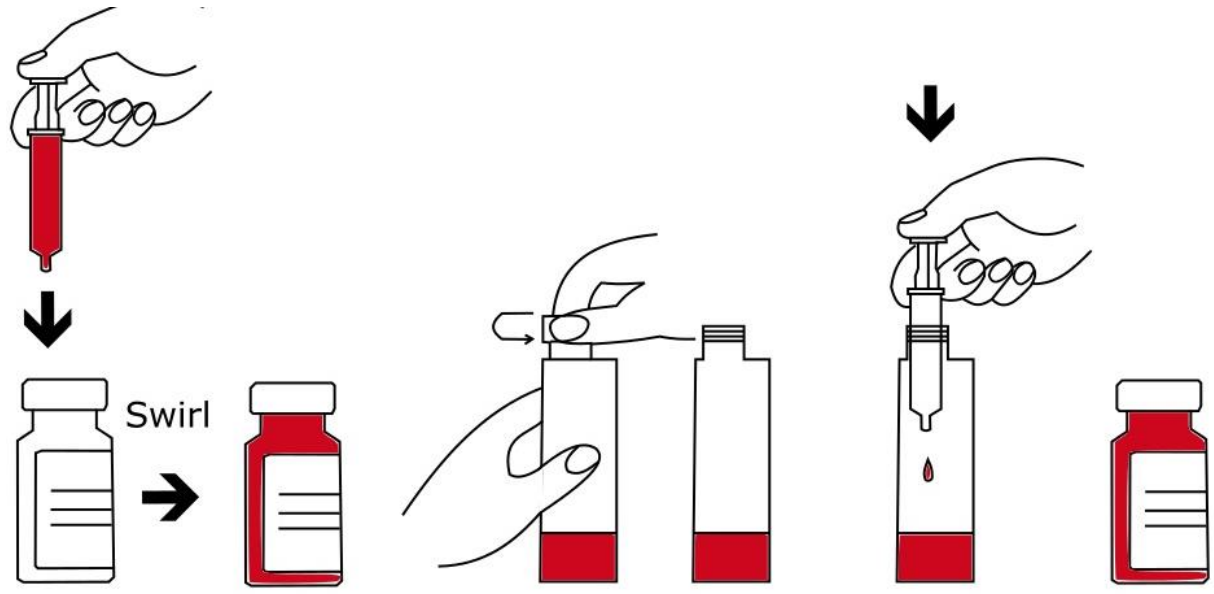
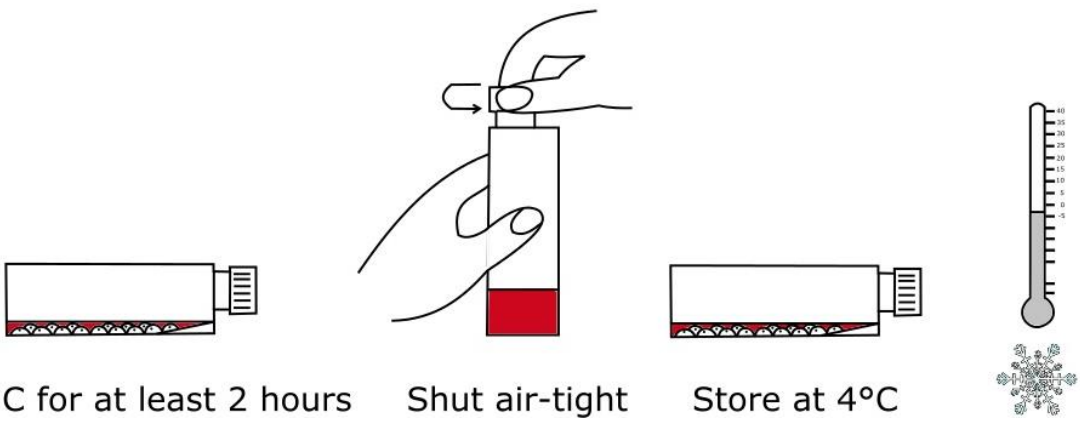
Application

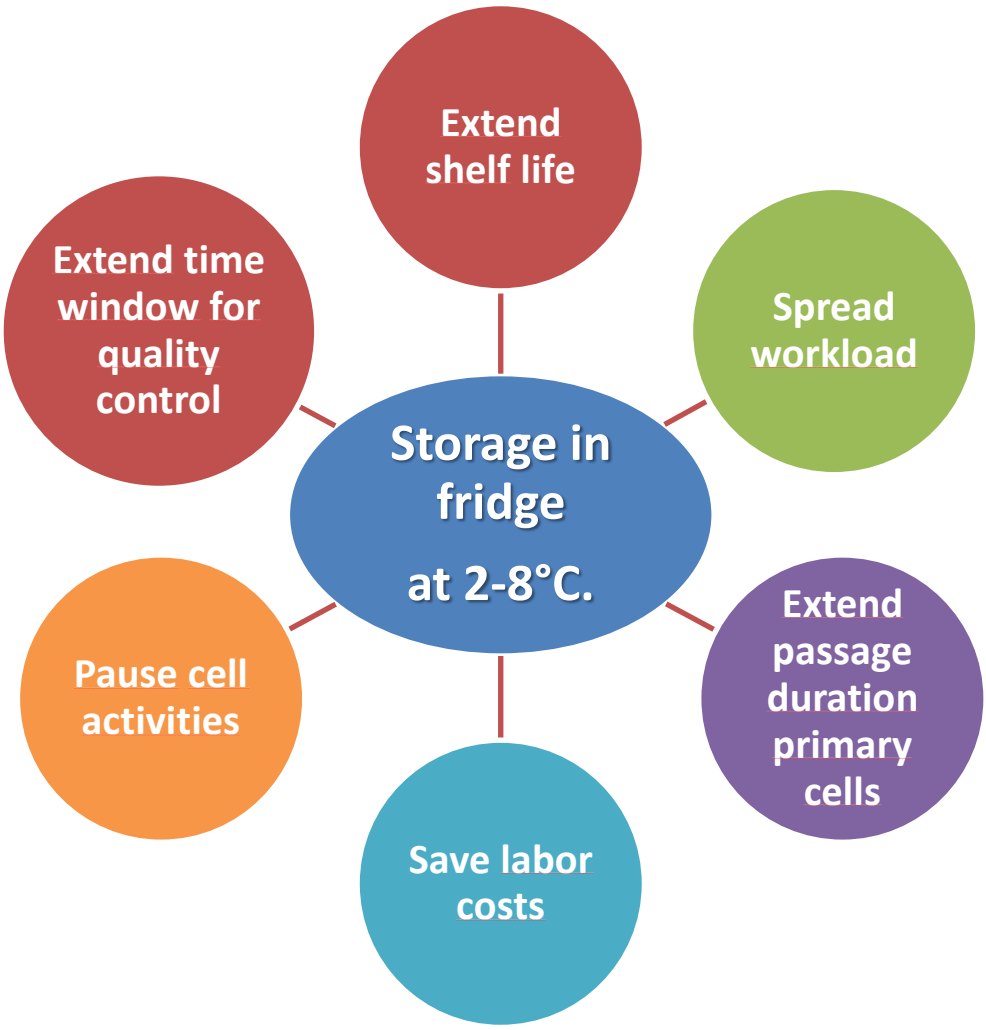


Create stock solution

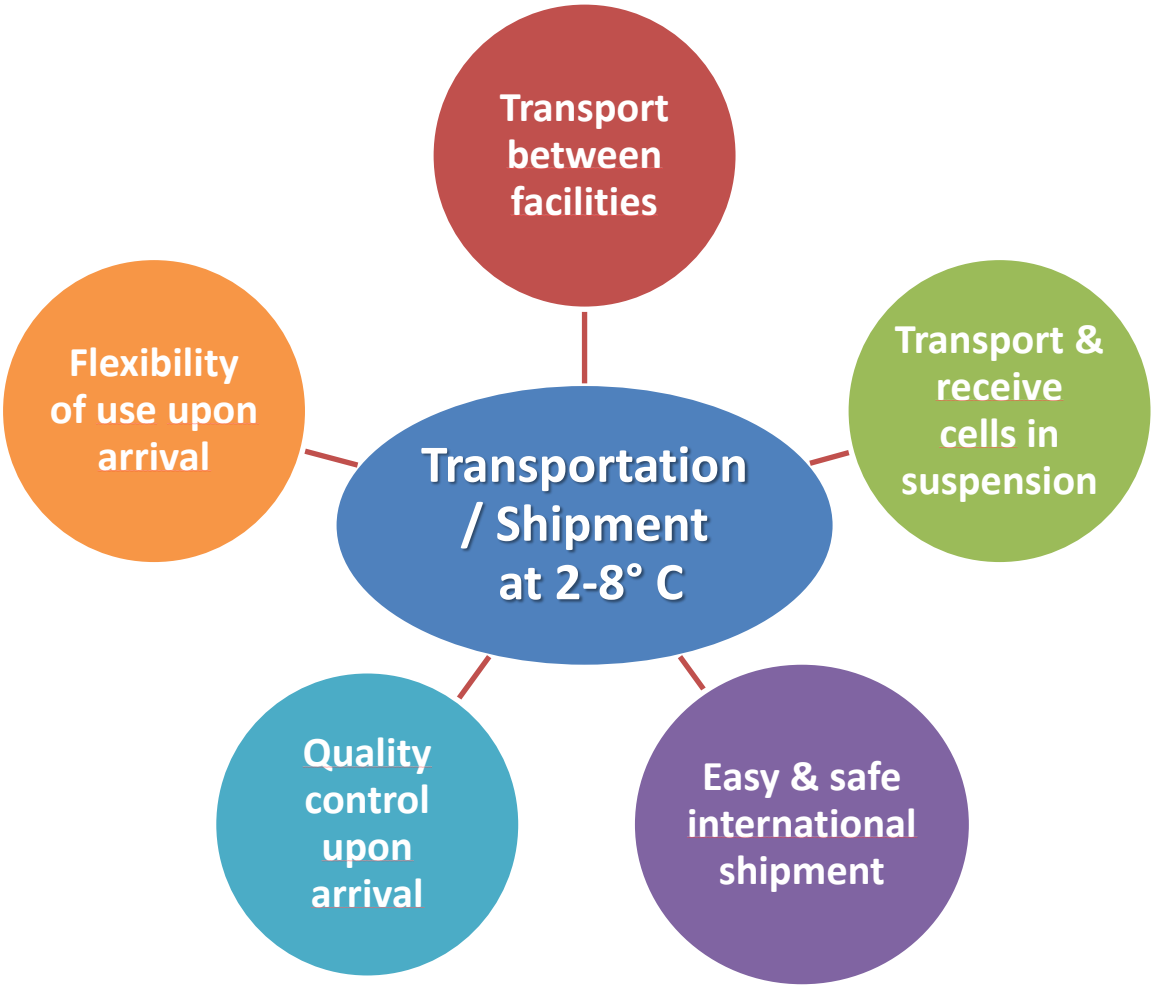


37°C for at least 2 hours | Shut air-tight | Store at 4°C

Use and benefits

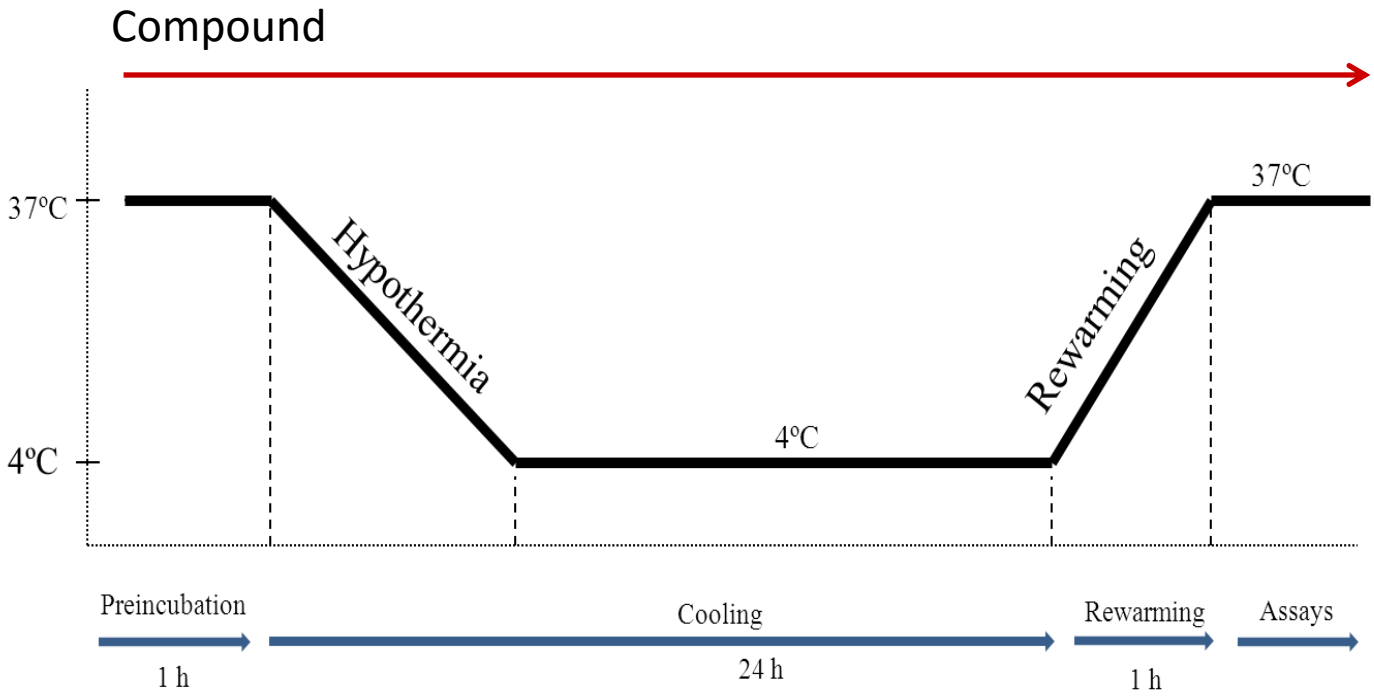


Use and benefits



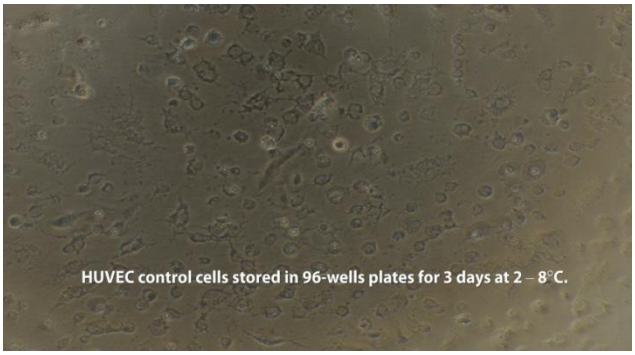
Hypothermia rewarming model

- Assess cell protective effect
- Read-outs:
  - Trypan blue exclusion (dead cells)
  - Neutral red uptake (viable cells)
  - Growth curve after storage at 2-8 °C

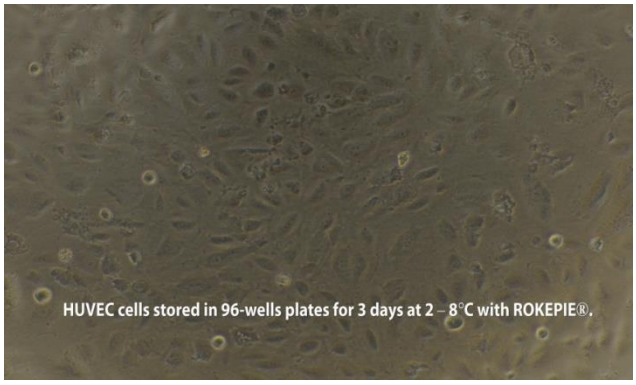


Tested primary cell lines

HUVEC control cells stored in 96-wells plate for 3 days at 2-8° C.

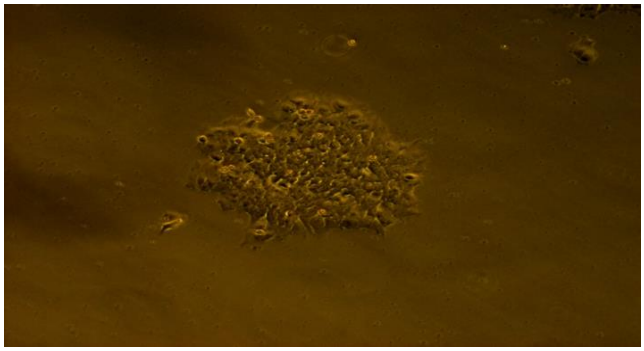


HUVEC cells stored in 96-wells plate for 3 days at 2-8° C with ROKEPIE®.



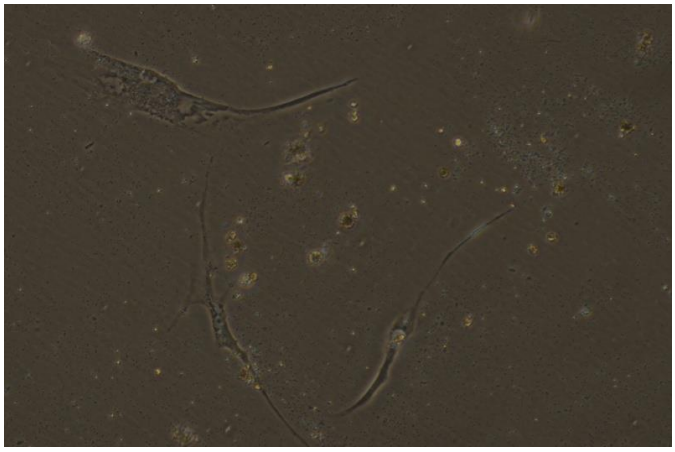
HUES9 control cells after storage in 6-wells plate for 7 days at 2-8° C.

HUES9 cells after storage in 6-wells plate for 7 days at 2-8° C with ROKEPIE®.

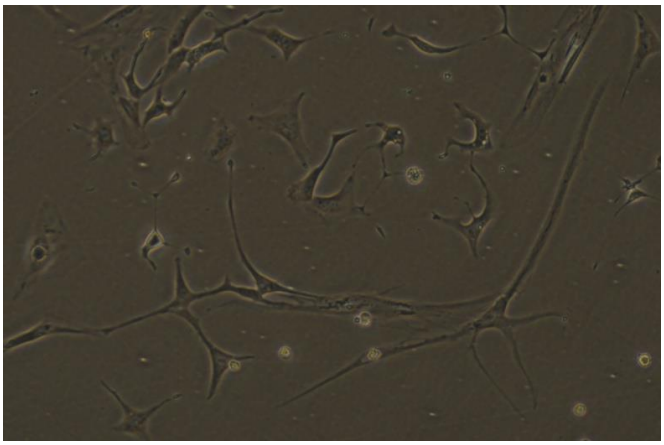


Tested cell lines (2)

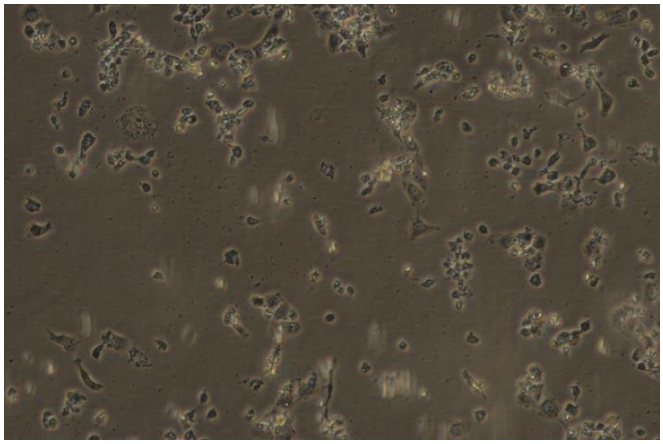
hADSC control cells stored for 11 days at 2-8° C.



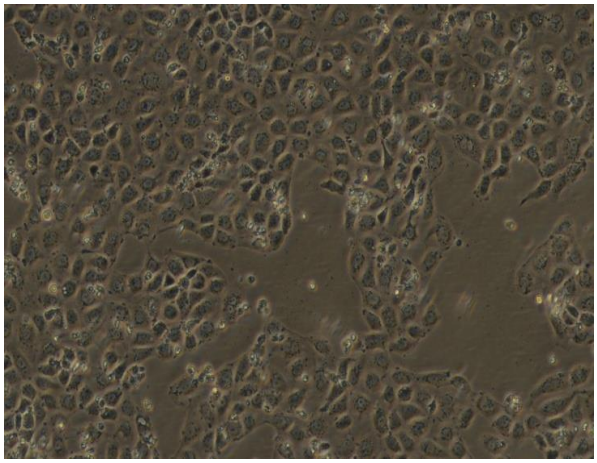
hADSCs stored for 11 days at 2-8° C with ROKEPIE®.



NRK 52E control cells after storage for 21 days at 2-8° C.



NRK 52E cells after storage for 21 days at 2-8° C with ROKEPIE®.



Overview test results

	Concentration	Storage duration (days)
Primary astrocytes	1:10	3
CaCo-2	1:10/1:100	7
HEK 293	1:10/1:100	7
HUVEC	1:10/1:100	13
MS5	1:10	7
N2A*	1:10/1:100 0.1/1/10 µM	7
NRK 52E	1:10/1:100	21
SH-Sy5y	1:10/1:50	7
hADSC	1:100	3
HDF*	1:20	2
THP-1*	1:25	2
Human skin punches*	1:10	5
Aorta rings rat*	100 µM	N.A.
Pigs heart*	10 mL/L	1

Interesting topics

- Cell culture media
  - Other than DMEM, RPMI, Ham's F-12
- Culture media composition
  - Serum, buffers
- Suspension cell culture
  - THP-1
- Functionality of cell lines
  - Differentiation, cytokine production
- Detachment of intact monolayer
  - As seen with HUVEC
- Passage number and cold tolerance

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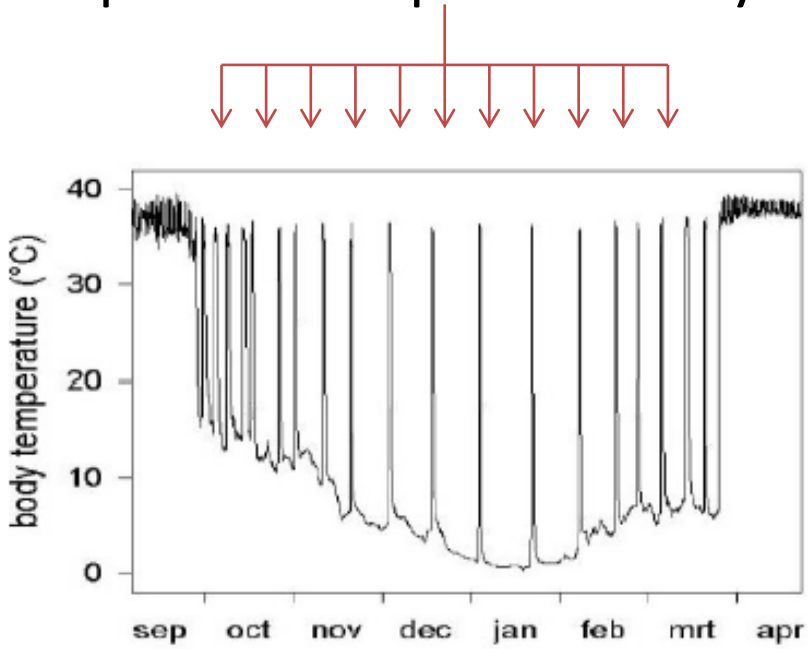
Background: hibernation technology

Natural resistance against damage

- Hibernation is nature's solution for surviving harsh times (food, temperature)
- Hibernators use a protective mechanism during hibernation

Interesting phenomena of hibernation

- No impact of fattening or cooling on health
- No signs of damage to organs or brain
- Active inhibition of metabolism – strong decrease in food and oxygen consumption
- Various adaptations to protect body and mind



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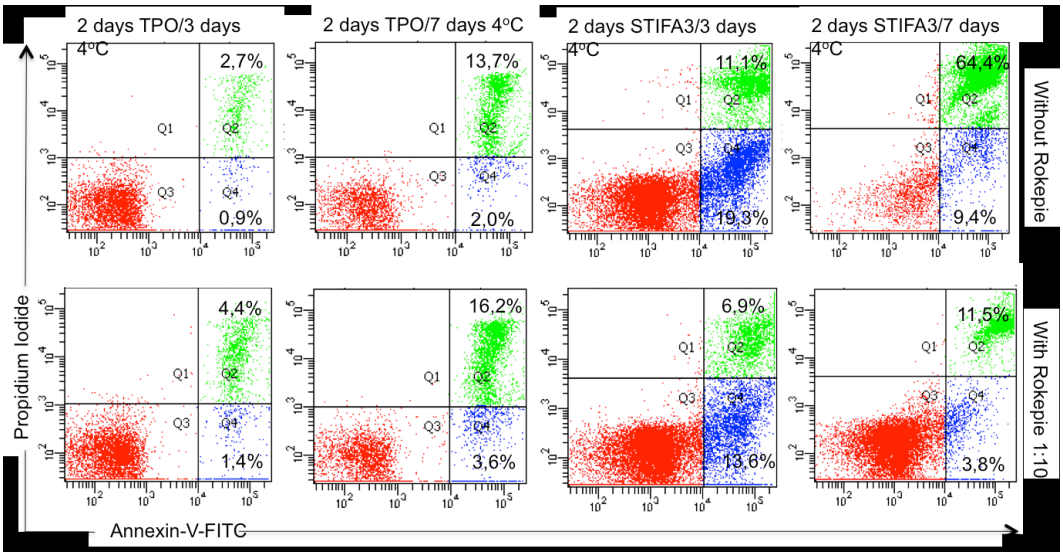
Users about ROKEPIE

European Society of Gene & Cell Therapy

"ROKEPIE *effectively promotes* cell viability of primary human hematopoietic stem cells during hypothermic storage"



P030

EUROPEAN SOCIETY OF GENE & CELL THERAPY

Optimization of methods for hypothermic preservation of primary human Hematopoietic Stem Cells in culture

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Introduction

Biogenic amines (dopamine and serotonin) protect cells against hypothermia-induced damage (1). The newly developed compound ROKEPIE®-S01 (Sulfateq BV) has been demonstrated to be successful in hypothermic storage and transportation of several cell lines, as well as thrombocytes (2), but not of primary cells, including hematopoietic stem cells (HSCs). Hypothermic preservation of HSCs could be preferred over short-term cryopreservation, to prevent cell loss during freezing/thawing and could be particularly useful for short-term storage, e.g., during conditioning of transplant patients or transport of HSC transplants. Here, we assessed optimal doses of this compound and culture conditions to preserve CD34+ umbilical cord blood (UCB) HSCs.

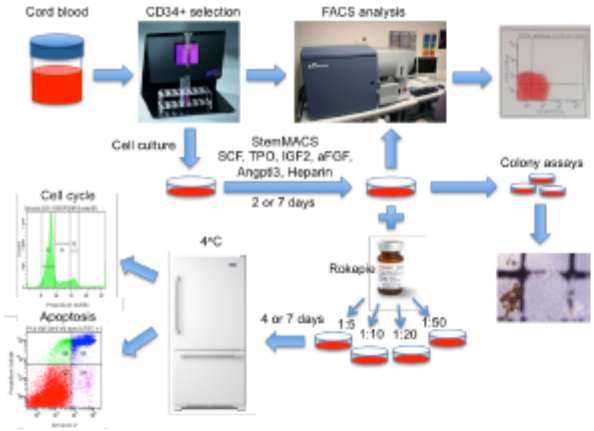

Figure 1. Prolonged hypothermic storage of Hematopoietic Stem Cells
To optimize conditions for prolonged hypothermic storage of HSCs, umbilical cord blood CD34+ cells were selected and cultured in presence of different growth factor cocktails, cultured for 2 or 7 days, supplemented without washing steps with Rokepie at dilutions of 1:5 – 1:50 and analyzed for colony forming units, CD34+ content, apoptosis (Annexin-V-FITC/Propidium Iodide) and cell cycle status.

Materials and Methods

CD34+ HSCs were isolated from freshly collected umbilical cord blood samples using magnetically labeled anti-CD34 antibodies (Miltenyi). HSCs were supplemented with TPO, TPO/SCF, TPO/SCF/Flt3L or TPO/SCF/aFGF/IGF2/Angptl3/heparin (STIFA3) for 2 days (analogous to our gene transfer method) or 7 days (analogous to our HSC expansion method). After 2 or 7 day cell culture, cell counts, colony forming assays (Methocult, Stem Cell Technologies) and CD34 cell numbers were assessed using a FACSARIA (Becton Dickinson). ROKEPIE®-S01 was then added directly to the medium, the plates closed airtight and stored for 4-7 days at 4°C. After rewarming, the frequency of apoptotic cells and cell cycle status were measured (Figure 1).

Results

Two day cultures with TPO resulted in the lowest frequency of apoptotic cells after hypothermic storage in the presence of 1:10 or 1:20 diluted ROKEPIE®S01. After 7 days of culture/hypothermic storage, cell viability was 10-30% higher in cultures containing the compound, whereas numbers of cycling cells were 10-20% lower, in particular when stimulated with STIFA3 (Figure 2).

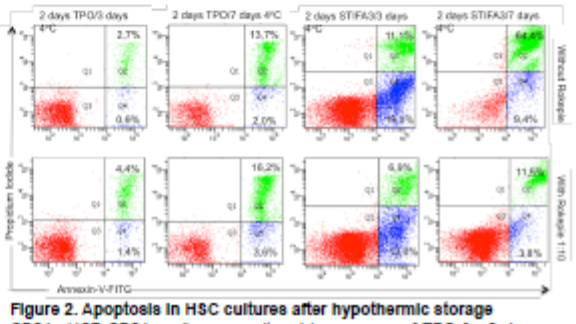

Figure 2. Apoptosis in HSC cultures after hypothermic storage
CD34+ UCB-CD34+ cells were cultured in presence of TPO for 2 days or STIFA3 for 7 days. ROKEPIE was added to the cultures and the flasks were closed air-tight and stored at 4°C for 3 or 7 days. Apoptosis was measured using Annexin-V-FITC and Propidium Iodide.

In short-term HSC cultures, followed by short-term storage at 4°C, ROKEPIE®S01 does not appear to affect cell viability or cell cycle state. Under long-term HSC expansion conditions, followed by 7 days of hypothermic storage, however, effects of the compound are significant, as evident from decreased rate of apoptosis, and increased levels of cells in G0/G1 phase (Table 1).

Table 1. Cell cycle analysis of HSC cultures after hypothermic storage

Cell cycle	G0/G1 (%)	S (%)	G2/M (%)	S+G2/M (%)
2 days TPO/3 days 4°C without Rokepie	12,2	11,7	11,8	23,5
2 days TPO/3 days 4°C Rokepie	15,1	15,2	15,0	30,2
2 day TPO/7 days 4°C without Rokepie	19,9	11,5	8,5	20,0
2 day TPO/7 days 4°C Rokepie	14,5	15,9	16,4	32,3
7 days STIFA3/3 days 4°C without Rokepie	30,5	27,3	23,9	51,2
7 days STIFA3/3 days 4°C Rokepie	27,7	26,5	26,6	53,1
7 days STIFA3/7 days 4°C without Rokepie	12,7	25,1	31,4	56,5
7 days STIFA3/7 days 4°C Rokepie	24,6	26,1	24,3	50,4

Conclusions

ROKEPIE®-S01 effectively promotes cell viability of primary HSCs during hypothermic storage, and is currently assessed for its effects on long-term repopulating HSC.

Rerences

Talaei F et al., PLoS One. 2011;6(7):e22568
Talaei F et al. Patent application WO2011128458 A1

Acknowledgements

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Users about ROKEPIE

“We like to share our encouraging results; When ROKEPIE® is used at 1/10 dilution and dispensed in both apical and basal wells of the plate, we see a major improvement. Without adding ROKEPIE® we simply cannot store and ship in cells in our gel formulation.’’

“We tested the ROKEPIE® with primary human keratinocytes, for 3 days at 4°C. We found that the cells recommenced normal proliferation after they were returned to the incubator. It is of interest for transport purposes, which we will investigate further.’’

“Thus far, I am quite satisfied with the results that we have been achieving using the ROKEPIE® hibernation reagent (especially also given the ease of its use) and I’d be happy to perform a few more experiments. ‘’

“We have tested ROKEPIE® in a 1:20 dilution added to our cell culture by storing them in the fridge for 24 hrs. The morphology after storage was very good, many mitoses were visible. The 3D model images after 10 and 14 days were very good, as well as the barrier function.’’

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Hibernation applied in life sciences

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