ROKEPIE®
HIBERNATION TECHNOLOGY FOR CELLS

Transport of fresh cell suspensions at 2 – 8°C

In this document, the protocol that we used to transport cell suspensions of freshly trypsinized cells at 2 – 8°C via regular post will be described. Cells can survive these low temperatures by adding ROKEPIE®-FD01 to the cell culture medium, whereas the low temperature prohibits cells from adhering to the plastic vessel and each other despite the fact that any cell dissociation reagent is already neutralized.

Multiple benefits

Storage and transportation of cells in suspension at 2 -8°C allows technicians and other laboratory personnel to transport or receive cells which are ready to be cultured or used upon arrival. This eliminates the need to remove toxic cryoprotectants or carefully dilute frozen cells to prevent a temperature- or osmotic shock.

An additional benefit of cell transportation at 2 – 8°C is the fact that dry ice is not needed for cooling, thereby reducing costs and eliminating the dangers of using dry ice. Preparation of cells for storage at 2 - 8°C does not require regulation of cooling rate, as is the case with cryopreservation of cells. For any additional information, please see our comparison of short- & long-term preservation methods at **http://www.rokepie.com/comparison-short-long-term-cell-preservation-methods/.**

Easy quality check of cells upon arrival

When cells arrive as a trypsinized suspension, it is easy to take a sample in order to determine viability. This can be used as a quality control check upon arrival, allowing for immediate feedback to the supplier once cells have arrived.

Additionally, the cells can immediately be cultured in any vessel: Once the preferred culture vessel (flask or plate) is determined, one vial of cells can be divided over several wells or all transferred to one flask in order to propagate. ROKEPIE®-FD01 supports growth at 37°C and does not need to be removed.

Summarized:

- ✓ ***Trypsinized cells remain in suspension at 2–8°C in cell culture medium***
- ✓ ***Cells survive short-term storage at 2–8°C with ROKEPIE®-S01***
- ✓ ***Transportation is safer and cheaper than using dry ice or liquid nitrogen***
- ✓ ***Immediate quality control upon arrival (viability) possible***
- ✓ ***Flexibility upon arrival: transfer to any vessel of preference (flask or plate)***

ROKEPIE®

sulfateq

Admiraal de Ruyterlaan 5
9725 GN Groningen
The Netherlands

T: +31 50 313 7464
E: info@rokepie.com
W: www.rokepie.com

Requirements

- 1 Expanded Polystyrene (EPS) box
- 1 Outside cardboard box
- 2 cooling elements at -20°C
- Any number of sterile 1,5 mL cups
- An adherent culture of your cells of interest
- Trypsine
- Cold cell culture medium (*e.g.* DMEM)
- 1 vial of ROKEPIE®-FD01

Protocol

The following protocol describes the necessary steps to achieve storage of trypsinized cells at 2 – 8°C, as well as how to proceed once cold trypsinized cells are received.

Preparing steps for transportation of cells:

1. Dissolve the lyophilized ROKEPIE-FD01 in 2.0ml solvent or cell culture medium of your choice.
2. Add ROKEPIE®-FD01 to your cells in their culture flask in the required dilution.
3. Pre-determine the necessary cell density for storage at 2 – 8 °C (see *Example* a).
4. Wash your cells with sterile PBS (free of Ca^{+} / Mg^{2+}) and trypsinize your cells.
5. Resuspend your cells in room temperature cell culture medium already containing ROKEPIE®-FD01, thus neutralizing the Trypsine.
6. Take a sample and determine the cell density on a hemocytometer or cell counter device.
7. In the meantime, gently shake the cell suspension to ensure homogenous distribution of cells.
8. Using the cell density, distribute the cell suspensions over the required number of sterilized Eppendorf cups (or vials), close them airtight and store at 2- 8 °C immediately.

Upon arrival:

1. Take the cells from their cold-storage container and place them in a fridge at 2- 8 °C.
2. Take a vial of cells and resuspend the cells carefully by pipetting.
3. Take a sample and mix 1:1 with Trypan blue.
4. Determine cell count and viability on a Hemocytometer.
5. If needed, dilute the cells in fresh cell culture medium and transfer the cells to the cell culture vessel of choice (plate or flask).
6. If preferred, replace the ROKEPIE®-FD01 supplemented cell culture medium in the flask or plate after about 4 hours of culture. By then, viable cells will have attached again.

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Results

Standard EPS box maintained low temperature for at least 36 hours

A regular EPS box, packed with 2 cooling elements, maintained temperatures <8°C throughout the experiment. In this experiment, even temperatures <2°C were obtained, indicating that 1 cooling element would suffice.

The cooling elements were taken from -20°C 45 minutes before packing to defrost the exterior. The cell samples were packed in bubble wrap, thus avoiding direct contact with the cooling elements.

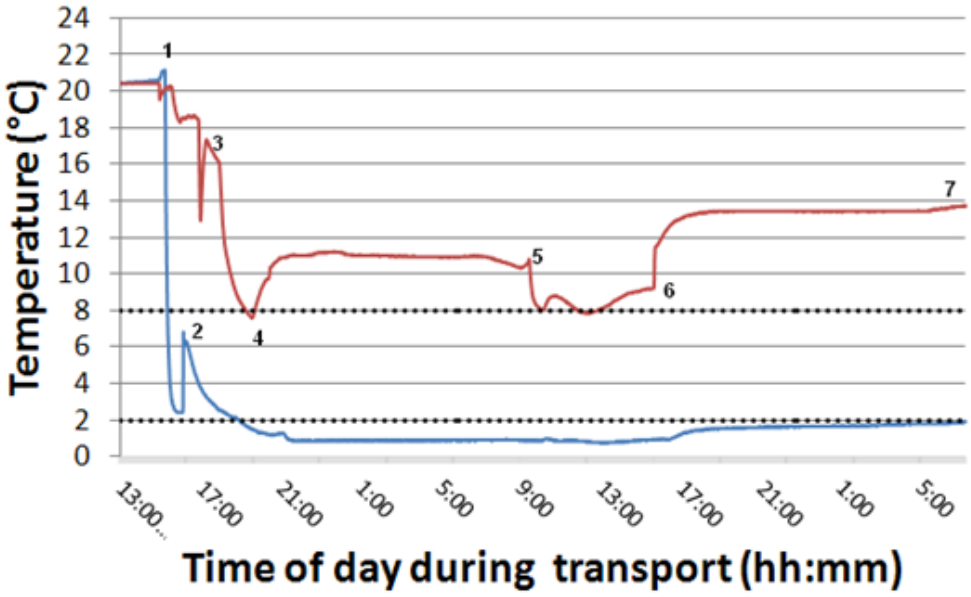

Figure 1 Temperature inside (—) and outside (—) the Expanded Polystyrene (EPS) box. **1**: Preparation of packaging, placement of cooling elements in EPS box, **2**: Final packaging, placement of samples in EPS box, **3**: Transfer to post office and subsequent transport from post office to sorting center, **4**: arrival at sorting center, overnight storage, **5**: transfer of package to delivery service, **6**: arrival at laboratory, overnight storage at room temperature, **7**: start of experiment, unpacking of box.

ROKEPIE®-FD01 safeguarded the viability of cells after transportation

Viability of HEK293 and 3T3-L1 cells was determined by Trypan blue exclusion on samples that were taken directly from the cups after opening the package. Even though temperatures <2°C were obtained, ROKEPIE®-FD01 increased viability of cells compared to control samples.

Note:

- ✓ ***For situations with high outside temperatures we advise to use an extra, small EPS box instead of the bubble wrap to keep the cells cool***
- ✓ ***Place the cells in the small EPS box, then place it in the larger EPS box and finally in the cardboard box***

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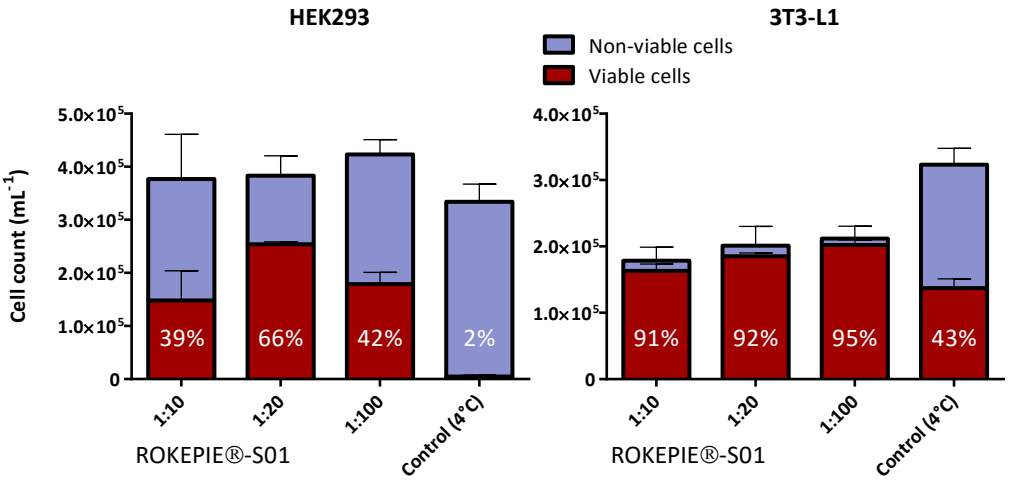

Figure 2: Viability of HEK293 cells (left) and 3T3-L1 cells (right) after ≤36 hours transport and storage at <8°C. Viability was determined by Trypan blue exclusion and is expressed as means +SD, 3 samples per condition.

Cell growth is maintained after storage of cells in suspension at 2 – 8°C

Cells that were cultured in 6- or 96-wells plates after storage as freshly trypsinized cells showed doubling times equal to those of 37°C control cells. After storage at 2 – 8°C, the first cells started adhere to culture vessels after about 30 minutes in warm cell culture medium (data not shown).

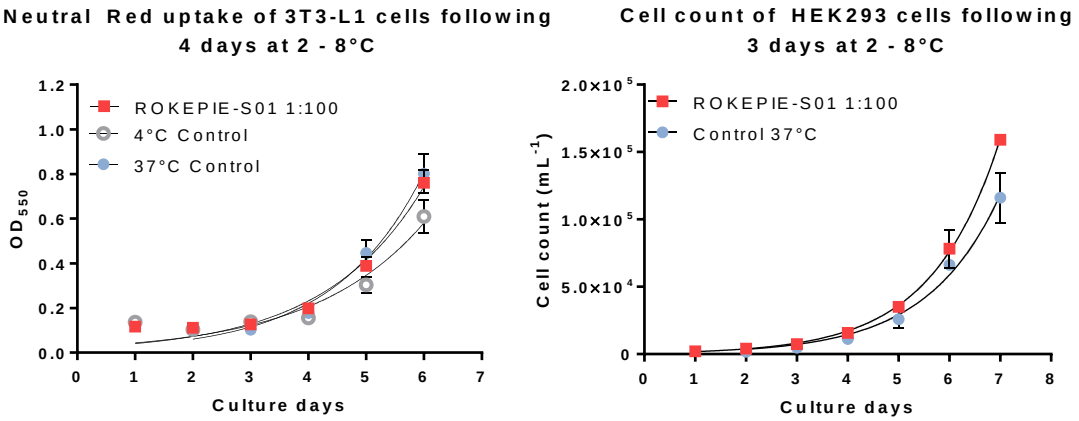

Figure 3. Examples of growth curves after cells have been stored as suspension at 2 – 8°C. Neutral Red uptake was used as a measure of viability in 3T3-L1 cells, whereas HEK293 cell count was determined by trypan blue exclusion. Data is shown as means (n=3) ± SD. These data originate from a separate experiment in which cells were stored as freshly trypsinized cells in a cold-storage room.

Summary

Since no dry ice or cold-chain transportation was necessary, we used a regular postal service. In the Netherlands, regular transport of a package of this size and weight (2 – 5 kg) costs €6,95.
In comparison to dry-ice shipment, this is a significant cut in costs. There were also additional benefits, such as flexibility upon arrival and the lack of need to remove toxic cryoprotectants.

If you have any questions about this experiment or protocol, please feel free to contact us.

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