

ROKEPIE®

HIBERNATION TECHNOLOGY FOR CELLS

Version: 2.0

Date: 12-sep-16

Hypothermic storage additive, lyophilized formulation

Product application/ intended use

ROKEPIE-FD01 is a supplement for your standard, well defined cell culture medium, enabling the hypothermic storage of cultured mammalian cells for several days. The lyophilized formulation should be dissolved in 2.0 mL solvent prior to use. This 2 mL of ROKEPIE-FD01 stock solution can be used to make 50 to 200 mL of hypothermic storage cell culture medium, depending on cell density and cell line in question.

About ROKEPIE-FD01

Research in the field of hibernation and its protective mechanisms led to the development of several novel chemical entities, designed to activate dormant protective mechanisms against the damage induced by hypothermia and subsequent rewarming. This product is designed to be used in your personal, well defined and cell-specific cell culture medium. Whereas other commercially available storage solutions completely replace cell culture medium, ROKEPIE-FD01 is simply an addition. Another benefit is that your cell culture medium immediately supports growth of viable cells after cold storage, without the need to remove the storage solution and apply fresh cell culture medium.

Storage and shipping

ROKEPIE-FD01 can be shipped and stored at room temperature. The container in which ROKEPIE-FD01 is delivered is meant to protect this product from light. Protect this product from direct light after reconstitution of the powder to prevent degradation of active substances. ROKEPIE-FD01 is delivered in a lyophilized formulation sufficient for 2.0 mL ROKEPIE-FD01 stock solution. The ROKEPIE-FD01 stock solution should be stored at 2 - 8 °C.

Directions for use

The following protocol is an example for use in adherent mammalian cells. The cell culture medium in this example uses bicarbonate as a pH-buffer. Cells are cultured in a flask with a standard phenolic style screw cap (i.e. without a membrane) which can be closed air-tight*. This enables 5% CO₂ enriched air to remain present in the flask during storage, preventing a sharp increase in pH due to a drop in CO₂.

1. Clean the outside of the container with your disinfectant of choice.
2. Dissolve the lyophilized ROKEPIE-FD01 in 2.0 mL solvent or cell culture medium of your choice.
3. Add the ROKEPIE-FD01 stock solution in a 1:25 ratio (confluent monolayer) or 1:50 ratio (semi con-fluent monolayer) to either fresh- or already present cell culture medium.
4. Incubate the cells in the supplemented medium for a period of = 2 hours at 37°C .
5. Close the flask air-tight and place the cells in a cold-room or refrigerator (4°C).
6. After cold-storage, place cells in a humidified incubator with a slightly opened cap and replace the supplemented cell culture medium once cells have re-attached**. This

normally takes about 6 – 24 hours. If cells have not detached during cold-storage, the supplemented cell culture medium can be replaced immediately.

You can consult the schematic representation of the directions for use on the backside of this folder.

*) If no standard phenolic style caps are available, the filter can be covered by parafilm in order to seal the flask air-tight. Wells or dishes can be covered entirely with parafilm, or all around the edges of the lid.

**) Detachment of cells can occur in some cell lines, but does not necessarily indicate they are apoptotic/ necrotic. ROKEPIE-FD01 is not toxic at working concentrations and 24 hour exposure to cells at 37°C should not result in loss of viability.


Warning and side notes

This product is for research use only. Even though this product has been tested on several cell lines (SMAC, HEK293, Skov-3, CaCo2), protection against the damage induced by hypothermia and rewarming by ROKEPIE-FD01 can be cell specific, and should therefore be tested on your cell-line of choice. It is recommended that the optimal duration of cold storage is determined per cell-line by the end-user.

Contact and questions

Please tell us about your experiences with this product. Any information about protection by ROKEPIE-FD01 is appreciated since this product is still in a research stage of development. Do you need more information? Please visit our website for our FAQ or contact us through:

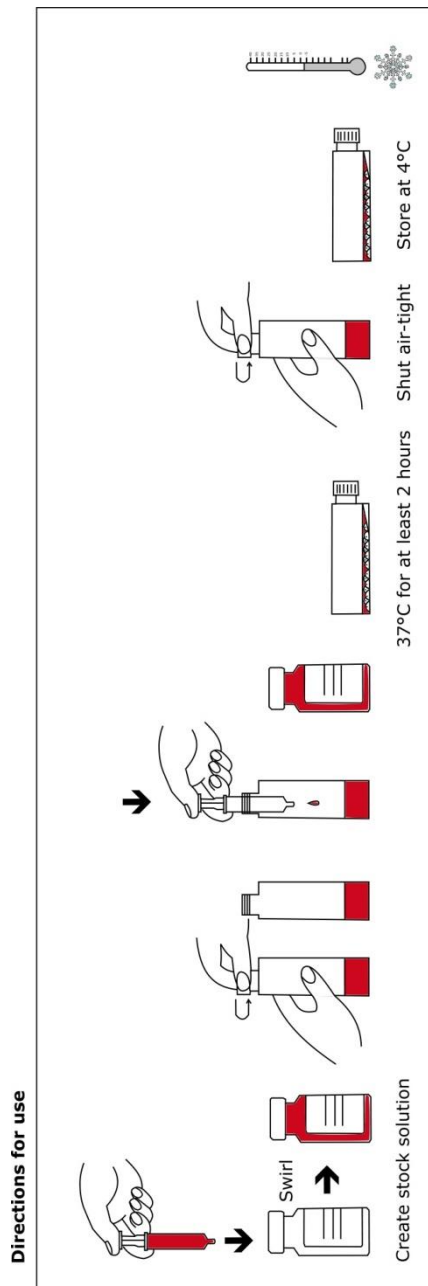
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The material must be handled as newly developed chemicals for research purposes with all needed precautions.

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Hypothermic Storage Solution Additive

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