Sprague-Dawley Rat Hepatic Stellate Cells
Catalog No. **RA-6242**

Suggested Medium: Complete Hepatic Stellate Cell Medium/w Kit – 500 ml
Catalog No. **M5569**

Product Description
Sprague-Dawley Rat Primary Hepatic Stellate Cells from *Cell Biologics* are isolated from liver tissues of 4-6 week old laboratory Sprague-Dawley rat. Cells are grown in tissue culture flasks and incubated in *Cell Biologics'* Cell Culture Medium for 2-5 days. Prior to shipping, cells at passage 0 are detached from flasks and cryopreserved in vials. Each vial contains $1x10^6$ cells per ml and is delivered frozen.

Product Testing
Sprague-Dawley Rat Primary Hepatic Stellate Cells from *Cell Biologics* are tested for expression of markers using antibodies, Desmin or α-actin by Immunofluorescence Staining. Cells are negative for bacteria, yeast, fungi, and mycoplasma. Cells can be expanded on a multiwell culture plate ready for experiments under the cell culture conditions specified by *Cell Biologics*. Hepatic Stellate Cells are not recommend for expanding or long term cultures since these cells would differentiate to become fibroblat-like cells immediately after plating and they will not proliferate in culture.

Laboratory Applications
Sprague-Dawley Rat Primary Hepatic Stellate Cells can be used in assays of standard biochemical procedures performed with cell cultures include RT-PCR, Western blotting, immunoprecipitation, immunofluorescent flow cytometry, or generating cell derivatives for desired research applications.

Storage of *Cell Biologics* Products
Cell Biologics ships frozen cells on dry ice. On receipt, immediately transfer frozen cells to liquid nitrogen until ready for experimental use. Live-cell shipment is also available on request. Primary cells can never be kept at -20 °C.

Authorized Uses of *Cell Biologics* Products
Sprague-Dawley Rat Primary Hepatic Stellate Cells from *Cell Biologics* are distributed for internal research purposes only. Our products are not authorized for human use, for *in vitro* diagnostic procedures, or for therapeutic procedures. Transfer or resale of any *Cell Biologics'* cells or products from the purchaser to other markets, organizations or individuals is prohibited by *Cell Biologics,* without the company's written consent. *Cell Biologics'* Terms and Conditions must be accepted before submitting an order.

Disclaimer
Investigators should handle the cells that they receive from *Cell Biologics* with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

Warranty and Liability
Cell Biologics' guarantee applies only to your purchase of *Cell Biologics'* cells with *Cell Biologics'* Media and Coating Solution, for appropriate cell culture and cell testing following *Cell Biologics'* online protocols within 35 days from the date of product delivery.

Primary Cell Culture Protocol

All cell culture procedures must be conducted in a bio-safety cabinet.
Any and all media, supplements, and reagents must be sterilized by filtration through a 0.2 µm filter.
Use aseptic technique to prevent microbial contamination.
Cryo-preserved cells must be stored in liquid nitrogen or seeded immediately upon arrival.

Medium:

Review the information provided on the *Cell Biologics'* website about appropriate culture media (e.g. serum and other supplements). Use pre-warmed (37°C) cell culture media (**30-50 ML**) to recover cryo-preserved cells and when changing media or splitting cells.

Coating of flasks or dishes:

Prepare a poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended) and leave the flask with 6 ml poly-L-lysine Coating Solution (*Cell Biologics*, Catalog No. 6951) in incubator overnight (minimum 2 hours at 37ºC incubator).

Aspirate the excess solution, Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

Cell recovery from cryovial:

- Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
- Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels.
- A seeding density of 20,000 cells/cm^2 is recommended.
- Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that stellate cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.
- Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
- Place the flask in a humidified, 5%-CO2 incubator at 37°C.
- For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be doubled after two to three days in culture.
- Cells should be checked daily under a microscope to verify appropriate cell morphology.

Please send us the cell images (>90% confluence) if you have any questions or problems with cultured cells.

Set up culture after receiving the order:
1. Prepare a poly-L-lysine coated flask (2 μg/cm^2, T-75 flask is recommended) and leave the flask in incubator overnight (minimum 2 hours at 37ºC incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 20,000 cells/cm2 is recommended. Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that stellate cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be doubled after two to three days in culture.