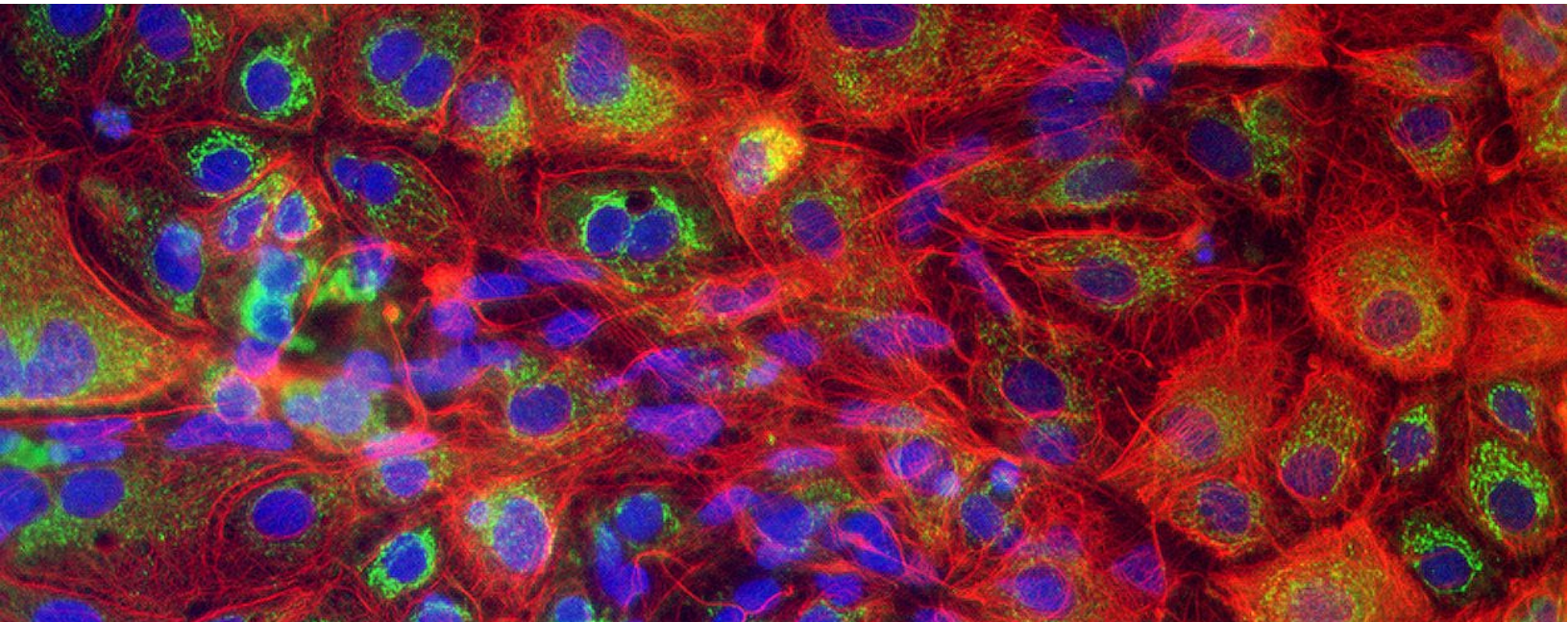




Hepatosight[®]-S User Guide



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Product Information

Unpacking & Handling

- ▼ Upon receiving the shipment of Hepatosight[®]-S, check whether all temperature-sensitive components are correctly frozen. If this is not the case, please contact our support team immediately.
- ▼ Immediately transfer each of the components to the appropriate storage conditions.
- ▼ Please check the catalog number, lot number and expiry date. The basal media expiration date is the shortest (1 year) so experiments should be planned accordingly.
- ▼ The Hepatosight[®]-S should be handled by technically qualified individuals complying with good laboratory practices, applicable laboratory regulations and the MSDS. Following the User Guide herein is recommended for best results.
- ▼ The Hepatosight[®]-S is intended for research use only, not intended for any type of use in animals or humans.

Components & Description

COMPONENTS	CAT#	STORAGE ON ARRIVAL
Hepatosight®-S Hepatocytes		Liquid Nitrogen
Cryopreserved, frozen vial		
>5 million cells	H-001	
>10 million cells	H-002	
Hepatosight®-S Media		-20°C
Basal medium		
70 ml	HM-001	
120 ml	HM-002	
Hepatosight®-S Media Supplement1		-20°C
0.7 ml (100X)	HS-001	
1.2 ml (100X)	HS-003	
Hepatosight®-S Media Supplement2		-20°C
0.6 ml (50X)	HS-002	
1.2 ml (50X)	HS-004	
Hepatosight®-S User Guide²		
Certificate of Analysis (CoA)		
MSDS³		

HM-001, HS-001&002 are used along with H-001, while HM-002, HS-003&004 are used along with H-002.

² Also available online at www.nexel.co.kr

³ Enclosed with shipping documents.

Should any of the above components be missing from your shipment, please contact us at NEXEL Co., Ltd. or the distributor in your country upon which our support team will provide the necessary assistance.

Hepatosight®-S Hepatocytes

Cell Type	Human Induced Pluripotent Stem Cell (hiPSC) derived Hepatocytes
Cell Line of Origin	hiPSC cell line reprogrammed from commercially available normal donor fibroblast cell line
Quality Control	Please refer to the CoA for lot-specific information. Virus clearance & STR analysis data is available upon request.

Hepatosight®-S Media & Media Supplement

- ▼ The Hepatosight®-S Media & Media Supplement 1 need to be combined to make the Hepatosight®-S Maintenance Media I, after which it should be used within 1 month. DO NOT FREEZE the Hepatosight®-S Maintenance Media I, aliquot into smaller quantities for best results.

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- ▼ The Hepatosight[®]-S Maintenance Media I & II are all serum-free. For additional information on the composition, please contact our technical support team.
- ▼ The Hepatosight[®]-S Maintenance Media is antibiotic and antifungal free as they are not necessary if proper conditions are kept. NEXEL does not recommend the use of such agents for accurate results but they should be used if aseptic cell culture conditions are not possible.

Safety Precaution & User Notice



Biosafety Level: 1

For research use only, not intended for any type of use in animal or humans. Appropriate safety procedures should always be used with this material. Please refer to the MSDS for detailed instructions.

User Notice & Restrictions:

- ▼ User may use the Product (Hepatosight®-S) for internal research including but not limited to screening potential drug compounds for efficacy and safety, and for the provision of such services to third parties. No other right is granted to User whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the Product does not include nor carry any right or license to use, develop or otherwise exploit the Product commercially, and no rights are conveyed to User to use the Product for any other purpose.
- ▼ User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans. Moreover, User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic or prophylactic purposes, or in animals for veterinary use for therapeutic, diagnostic or prophylactic purposes, including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine without an appropriate license.
- ▼ In the case that User transfers Product to a third party, User shall convey the User Restrictions set forth herein to such third party.

1. Introduction

NEXEL Co., Ltd. strives to provide high quality human Hepatocytes derived from induced pluripotent stem (iPS) cells using optimized proprietary protocols. The Hepatosight[®]-S is a highly pure and functionally active population of cells, ensuring researchers get a reliable product. Thus, NEXEL hopes to help the advance of science in tissue-specific research, toxicity screening and drug discovery.

This User Guide will help you seed the Hepatosight[®]-S at the appropriate densities to create homogenous layers of hepatocytes appropriate for a variety of applications related to the metabolic activity such as albumin secretion, glycogen production or CYP activity. However, please keep in mind that the best individual results will be obtained by close observation, care and optimization from the user.

2. Preparing for Cell Culture

Required Equipment and Consumables (Not Provided)

ITEM	CAT#	VENDOR
Coating Material		
Matrigel, hESC-qualified	354277	Corning
Collagen I		
Rat Tail	A1048301	Gibco
Typical Cell Culture Equipment		
Liquid Nitrogen Storage Tank		
37°C Water Bath		
Tabletop Centrifuge		
Biological Safety Cabinet with UV Lamp		
Hemocytometer or Automated Cell Counter		
Phase Contrast Microscope		
Pipettes		
Cell Culture Incubator		
Typical Cell Culture Consumables		
Centrifuge Tubes		
Cell Culture Plates		
Pipette Tips		
Trypan Blue		
Phosphate Buffered Saline (PBS)		

Preparing Hepatosight®-S Media

- I. Thaw the Hepatosight®-S Media by placing it at 4°C 24 hours prior to use.
- II. In a biosafety cabinet, thaw and add the Hepatosight®-S Media Supplement 1 (100X) to the thawed media to make Hepatosight®-S Maintenance Media I. Store at 4°C for up to 1 month. DO NOT FREEZE Hepatosight®-S Maintenance Media I.

To avoid oxidation of the media due to air contact and repeated warming/opening, it is recommended to aliquot the media into quantities enough for 2~3 media changes.

- III. Hepatosight®-S Maintenance Media I should be prepared immediately before plating the cells for best results.
- IV. From Day 3 to 7, Hepatosight®-S Maintenance Media II is required and should be made fresh. Thus, Hepatosight®-S Media Supplement 2 (50X) should be thawed on that day and then kept at 4°C. Add the supplement to the media aliquoted and warmed for that day's experiments.

MEDIA TYPE	COMPONENTS
Hepatosight®-S Maintenance Media I	Hepatosight®-S Media Hepatosight®-S Media Supplement 1 (100X)
Hepatosight®-S Maintenance Media II	Hepatosight®-S Maintenance Media I Hepatosight®-S Media Supplement 2 (50X)

Preparing Cell Culture Surfaces

- I. Calculate the amount of coating media required using the following table as a reference.

CELL CULTURE PLATE	6-well (9.6 cm ²)	12-well (3.8 cm ²)	24-well (1.9 cm ²)	48-well (1.0 cm ²)	96-well (0.33 cm ²)
COATING VOLUME	1.5 ml	1000 µl	500 µl	200 µl	100 µl

- II. Prepare the coating media to working concentrations immediately before use by mixing the components in PBS as described in the following table.

COATING TYPE	STOCK CONCENTRATION	WORKING CONCENTRATION
Matrigel	NA, 100X	1X
Collagen I	3 mg/ml	40 µg/ml

It is possible to use only Collagen I for the coating. Please contact the support team for more information.

- III. Pipette the correct amount of coating solution to each well you intend to use.
- IV. Gently swirl the plate and check whether all the wells are completely covered.
- V. Incubate at 37°C for at least 30 minutes.

3. Hepatosight®-S Thawing and Plating

Thawing

The Hepatosight®-S can be thawed using typical cell culture thawing protocols. Here, we present NEXEL’s optimized protocol and recommend our users to follow the instructions to maximize results. We strongly recommend thawing 1 vial at a time to minimize cell exposure to liquid DMSO.

- I. Calculate the amount of Hepatosight®-S Media and Maintenance Media I required. For each vial, 9 ml of Hepatosight®-S Media is required. The amount of Maintenance Media I required can be calculated by the number of wells and an additional 2ml per vial for resuspending the cells; recommendations for different cell culture plates can be found below.

CELL CULTURE PLATE	6-well (9.6 cm ²)	12-well (3.8 cm ²)	24-well (1.9 cm ²)	48-well (1.0 cm ²)	96-well (0.33 cm ²)
PLATING VOLUME	2 ml	1 ml	500 µl	300 µl	200 µl

- II. Warm the Hepatosight®-S Media and Maintenance Media I at Room Temperature (RT, 25°C) for at least 30 mins. For each vial to thaw, aliquot 8 ml of Hepatosight®-S Media in a 15 ml centrifuge tube.
- III. Retrieve the Hepatosight®-S vial(s) from the liquid nitrogen storage tank.
- IV. Submerge the vial(s) 2/3 in a 37°C water bath so that the mouth of the vial does not come in contact with the water. Constantly check how much has thawed and once ~20% remain (~3 mins), spray the vial(s) with 70% Et-OH, wipe and place it in your biosafety cabinet. Ideally, the vial(s) should have completely thawed exactly when you start step V.
- V. Open the vial(s) and transfer the contents (~1 ml) using a 1 ml pipette to the aliquoted 8 ml of Hepatosight®-S Media dropwise while gently swirling the tube.

Dropwise pipetting while gently swirling the tube minimizes osmotic shock and maximizes mixing, which ensures high viability. Drops will remain on the surface for ~1 second and then drop towards the bottom of the tube (visible due to the DMSO content). For dropwise pipetting, simply pipette slowly into the air ~1 cm above the media surface. It should take approximately 1 min per 1 ml.

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- VI. Use 1 ml of Hepatosight®-S Media to gently rinse the emptied vial and transfer dropwise to the centrifuge tube containing the cells from step V while gently swirling the tube.
- VII. Centrifuge the suspended cells at 180 x g for 3 minutes at room temperature.
- VIII. Carefully discard the supernatant.
- IX. Resuspend the cells gently using 2 ml of Maintenance Media I and check the cell concentration using a hematocytometer or cell counter and Trypan Blue. Immediately move on to the Plating section.

Avoid rigorous pipetting of the cells to maximize viability. Single cell resuspension of the Hepatosight®-S during thawing should easily be achieved by gently pipetting 3~4 times.

Plating

NEXEL recommends seeding the Hepatosight®-S at a density of ~250,000 cells/cm² for most standard applications. Application specific protocols are available upon request (will be added to the website in early 2020). The best results are obtained by the User's own optimization, for which NEXEL will try to provide as much assistance as possible.

- I. Calculate the volume of Maintenance Media I and cells required to match the correct density for the culture platform of choice. Below is a table with cell numbers.

CELL CULTURE PLATE	6-well (9.6 cm ²)	12-well (3.8 cm ²)	24-well (1.9 cm ²)	48-well (1.0 cm ²)	96-well (0.33 cm ²)
PLATING VOLUME	2 ml	1 ml	500 µl	300 µl	200 µl
CELL NUMBER	2,400,000	1,000,000	500,000	250,000	100,000

Well area (cm²) can vary between different vendors, please check with your providers for exact calculations.

- II. Combine the volumes as calculated above.
- III. Remove the coating solution in the cell culture plates. Avoid drying out the coated wells as much as possible.
- IV. Gently mix by pipetting and evenly distribute the appropriate volumes of cells with Maintenance Media I.
- V. Move the cell culture plate to the incubator, shake the plate in perpendicular directions to evenly distribute the cells for attachment.
- VI. The day after, perform the first media change with Maintenance Media I (Hepatosight®-S Maintenance Step I)

4. Hepatosight®-S Maintenance

Starting on the day after plating the cells, the media needs to be changed every two days. Ideally, the media should be changed at 48-hour intervals. Depending on the culture day either Hepatosight®-S Maintenance Media I or II are used. On days 1 & 9, use Hepatosight®-S Maintenance Media I. On days 3, 5 & 7, use Hepatosight®-S Maintenance Media II.

- I. Warm the correct volume of Hepatosight®-S Maintenance Media volume (same as for plating) at room temperature for at least 30 mins.
 - a. On days 3, 5 & 7, add the Hepatosight®-S Media Supplement 2 to the warmed Maintenance Media I at 50X.
- II. Replace the cell culture media with the newly warmed media in a biosafety cabinet. Pipette onto the cell culture plate walls to avoid any damage to the cell culture.
- III. Place the plate back in the incubator.
- IV. Repeat I to III every 2 days.

We recommend performing any planned assays with the Hepatosight®-S from Day 7 onwards. Long term culture over 11 days can lead to loss of morphology or function, we recommend modifications to culture methods such as organoid culture to overcome these issues.