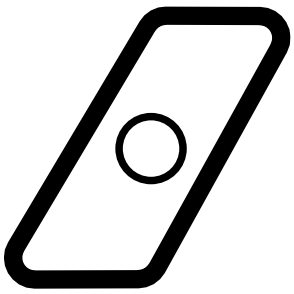
NEXEL

Hepatosight®-S
User Guide



Contents

1. Product Information 2
    Unpacking & Handling 2
    Components & Description 3
    Safety Precaution & User Notice 5
2. Introduction 6
3. Preparing for Cell Culture 7
    Required Equipment and Consumables (Not Provided) 7
    Preparing Hepatosight®-S Media 8
    Preparing Cell Culture Surfaces 9
4. Hepatosight®-S Thawing and Plating 10
    Thawing 10
    Plating 12
5. Hepatosight®-S Maintenance 13

1. Product Information

Unpacking & Handling

- Upon receiving the shipment of Hepatosight®-S, check whether all temperature-sensitive components are correctly stored. 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 (date of expiration on label) 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		
Cryopreserved, frozen vial		Liquid Nitrogen
>5 million cells	H-001	
>10 million cells	H-002	
Hepatosight®-S Media		
Maintenance medium		4°C
70 ml	HM-001	
100 ml	HM-002	
Hepatosight®-S Maintenance Supplement		-20°C
0.7 ml (100X)	HS-001	
1.2 ml (100X)	HS-002	
Hepatosight®-S User Guide[1]		
Certificate of Analysis (CoA)		
MSDS[2]		

[1] Also available online at www.nexel.co.kr

[2] 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 Advanced Media & Media Supplements

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

- The Hepatosight®-S Maintenance Media are serum-free. For additional information on the composition, please contact our technical support team.
- The Hepatosight®-S Maintenance Media are 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.

2. 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 homogeneous layers of Hepatocytes appropriate for a variety of applications related to the metabolic activities 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.

3. Preparing for Cell Culture

Required Equipment and Consumables (Not Provided)

ITEM	CAT#	VENDOR
Coating Material		
Matrigel	354234	Corning
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

1. Thaw the Hepatosight®-S Media and Supplements by placing them at 4°C 24 hours prior to use.
2. In a biosafety cabinet, thaw and add the Hepatosight®-S Maintenance Supplement (100X) to the thawed medium to make corresponding Media. Store at 4°C for up to 1 month. DO NOT FREEZE Hepatosight®-S Maintenance Media.
    - 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.
3. Hepatosight®-S Maintenance Media should be prepared immediately before plating the cells for best results.

MEDIA TYPE	COMPONENTS
Hepatosight®-S Maintenance Media	**Hepatosight®-S Maintenance medium** **Hepatosight®-S Maintenance Supplement (100X)**

Preparing Cell Culture Surfaces

1. 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 ml	500 µl	300 µl	200 µl	100 µl

2. Prepare the coating solution to working concentration immediately before use as described in the following table. Coating solution can be kept at 4°C for a short period of time but such is not recommended.

COATING TYPE	STOCK CONCENTRATION	WORKING CONCENTRATION
Matrigel	**NA, 100X**	**1X (15 µl/ml)**

- Collagen I is also a viable option to be used as coating material. If Collagen I is used, make sure to thoroughly wash the cell culture plate surface before seeding.

3. Pipette the correct amount of coating solution to each well you intend to use.
4. Gently swirl the plate and check whether all wells are completely covered.
5. Incubate at 37°C CO_2 Incubator for more than 1 hour.

4. 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.

1. Calculate the amount of Hepatosight®-S Maintenance Media required. For each vial, 10 ml of Maintenance Media is required for resuspending the vial. The amount of Maintenance Media required can be calculated by the number of wells; 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

2. Warm the Maintenance Media at Room Temperature (RT, 25°C) for at least 30 mins. For each vial to thaw, aliquot 8 ml of Maintenance Media in a 15 ml centrifuge tube.
3. Retrieve the Hepatosight®-S vial(s) from the liquid nitrogen storage tank.
4. 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 5.
5. Open the vial(s) and transfer the contents (~1 ml) using a 1 ml pipette to the aliquoted 8 ml of Maintenance 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.
6. Use 1 ml of Hepatosight®-S Maintenance Media to gently rinse the emptied vial and transfer dropwise to the centrifuge tube containing the cells from step 5 while gently swirling the tube.
7. Centrifuge the suspended cells at 180 x g for 3 minutes at room temperature.

8. Carefully discard the supernatant.
9. Resuspend the cells gently using 1 ml of Maintenance Media 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/cm^2 for most standard applications. Application specific protocols are available upon request. Best results are obtained by the User's own optimization, for which NEXEL will try to provide as much assistance as possible.

1. Calculate the volume of Maintenance Media 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	2,500,000	900,000	450,000	250,000	100,000

Well area (cm^2) can vary between different vendors, please check with your providers for exact calculations.

2. Combine the volumes as calculated above.
3. Remove the coating solution in the cell culture plates. Avoid drying out the coated wells as much as possible.
4. Gently mix by pipetting and evenly distribute the appropriate volumes of cells with Maintenance Media.
5. Move the cell culture plate to the incubator, shake the plate in perpendicular directions to evenly distribute the cells for attachment.
6. The day after, perform the first media change with Maintenance Media (Hepatosight®-S Maintenance Step 1).

5. Hepatosight®-S Maintenance

Starting 24 hours after plating the cells, the media needs to be changed every two days. Ideally, the media should be changed at 48-hour intervals. When performing functional assays on the cells, we recommend changing the media on the morning of the experiment to ensure there are enough nutrients for the cells and to deliver the desired drug concentration.

1. Warm the correct volume of Hepatosight®-S Maintenance Media at room temperature for at least 20 minutes.
2. Perform a media change with the newly warmed media in a biosafety cabinet. Pipette softly onto the cell culture plate walls to avoid any damage to the cell culture.

   - 24 hours after plating, replace a full volume of existing Media with Maintenance Media. To remove the spent media, aspirate the media using a pipette without tilting the plate. Leave a small amount of media so that the cells do not come in contact with air. Then gently add appropriate volume of pre-warmed Maintenance Media.

   - After the initial full-media change, perform full-media changes on 48-hour interval.

   - While performing media changes, it is critical to keep the plate flat on a surface. Tilting the plate during half-media changes can cause the cells to shift position and potentially induce aggregation towards the tilted direction.

3. Place the plate back in the incubator.
4. Repeat 1 to 3 every 2 days.

We recommend performing any planned assays with the Hepatosight®-S from Day 10 onwards.