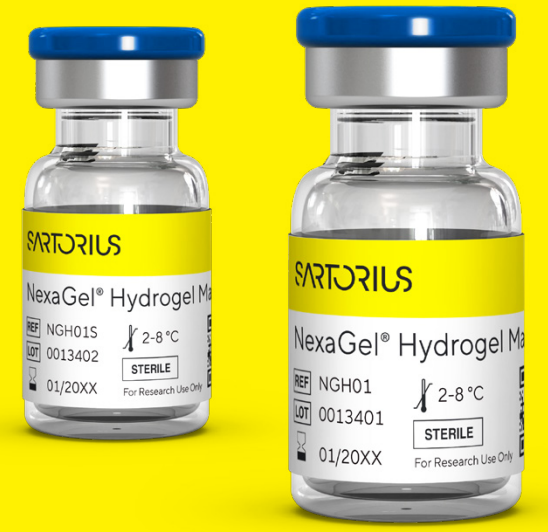
Instructions For Use

Original Instructions For Use

NexaGel® Hydrogel Matrix

NGH01 | NGH01S

Hydrogel for cell culture research



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Content

1 About These Instructions

1.1 Validity

These instructions are part of the product; they must be read in full. These instructions apply to the product in the following versions:

Product	Article number
NexaGel® Hydrogel Matrix, 10 mL	NGH01
NexaGel® Hydrogel Matrix, 2 mL	NGH02S

1.2 Related Documents

▶ In addition to these instructions, observe the following documents:
- Safety Data Sheet (SDS) for the product
- Instructions for Use of other solutions used

1.3 Target Groups

These instructions are addressed to the following target groups. The target groups must possess the knowledge specified below.

Target group	Knowledge and Qualifications
User	The user is familiar with the product and the associated work processes. The user understands the hazards which may arise when working with the product, and knows how to prevent them.

1.4 Symbols Used

▶ Required action: Describes activities that must be carried out. The activities in the sequence must be carried out in succession.

▷ Result: Describes the result of the activities carried out.

2 Safety Instructions

2.1 Intended Use

NexaGel® Hydrogel Matrix is a xeno-free (animal origin-free) hydrogel that is intended for cell culture research, especially for 3D cell culture research (see Chapter “5.1 Formulation and Use”, page 11).

The product transforms into a hydrogel matrix by mixing with the cell culture medium. **No** cross-linking agents are required.

The product is suitable for injection and provides a solution for the entire research pipeline from initial cell line tests to *in vivo* studies using the same platform system.

The product is intended for research use only. It is **not** intended for use in diagnostic procedures. The product is restricted to professional users.

The product is intended solely for use in accordance with these instructions. Any further use beyond this is considered improper.

2.2 Precautions

Read the Safety Data Sheet (SDS) before using the product. The SDS includes instructions for safe handling, storage, and disposal of the product.

3 Product Description

3.1 Overview

The hydrogel is a ready to use product. The product is formulated with a precise combination of multi-functional ligands and concentrations, making it suitable for a wide variety of cell types and applications.

The product effectively mimics the natural extracellular matrix (ECM), creating a more familiar environment for cells. Cells grown can be easily retrieved using a cell recovery solution.

3.2 Culture Methods

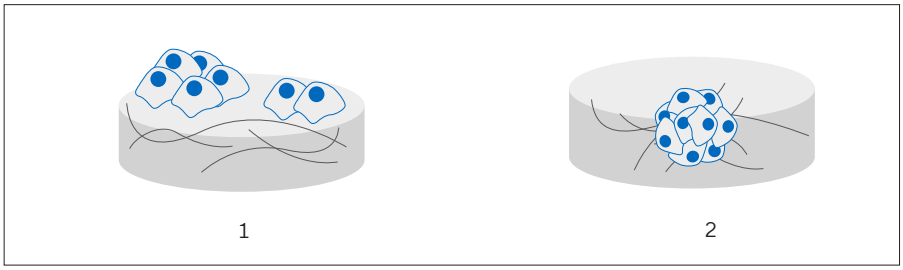

Fig. 1: Overview of culture methods

Pos.	Name	Description
1	2D cell culture	To control the substance properties for culturing cells on top of the hydrogel. Especially suitable for cell submergence or invasion studies.
2	3D cell culture	To encapsulate cells in the hydrogel matrix to promote cell-matrix and cell-cell interactions.

3.3 Cell Harvesting

Harvesting of cells for downstream analysis is possible by using a cell recovery solution (see Chapter “5.3 Hydrogel Application”, page 11). With the cell recovery solution the hydrogel rapidly breaks down, leaving a cell suspension that can be used for cytometry, molecular analysis methods, or continued cell culture.

4 Protocol

4.1 NexaGel® 2D Cell Culture Protocol

4.1.1 Pre-coating the Culture Vessel

Pre-coating the well plate before adding the hydrogel and seeding cells helps to minimize 2D cell growth on the bottom of the well which can interfere with imaging and analysis.

Multiple coatings can be used including poly-D-lysine (PDL) and poly-L-ornithine (PLO). This protocol uses PLO as a coating solution.

Materials:
- Coating solution, e.g., poly-D-lysine (PDL) and poly-L-ornithine (PLO)
- CO_2 incubator

Procedure

- ▶ Dilute the PLO solution to the desired concentration using tissue culture grade water or PBS.
- ▶ Add the diluted PLO solution to the plate, making sure to cover the entire surface. The required volume depends on the size of the culture vessel, e.g., 75 µL for a 96-well plate.
- ▶ Incubate the plate at room temperature for 1 hour.
- ▶ Aspirate the excess PLO solution and rinse the wells with PBS.
- ▶ Dispense the appropriate volume of hydrogel into each well.

4.1.2 Preparing the Hydrogel and Cell Seeding

Procedure

- ▶ Bring the hydrogel to room temperature or warm to 37 °C.
- ▶ Dilute the hydrogel with cell culture medium in the desired concentration:
  - ▶ We recommend to dilute the hydrogel 2:1 with cell culture medium.
  - ▶ If using cell culture medium with low salt concentration such as RPMI 1640 medium: Consider using 1:1 v/v mixing ratio.
  - ▶ To maintain the correct concentration of growth factors, serum, or other media additives: Add these supplements to the hydrogel at 3X concentration to counteract the dilution effect and achieve a final concentration within the hydrogel of 1X.
- ▶ Gently pipette up and down 5-10 times to mix thoroughly and avoid introducing bubbles.
- ▶ Dispense the appropriate volume of hydrogel into each well (for the recommended volumes see Chapter “5.5 Recommended Volumes for Hydrogel”, page 12).
- ▶ Gently swirl the well plate to ensure an even covering on the bottom of each well and remove bubbles using ethanol vapor.
- ▶ Allow the hydrogel to polymerize for 15 minutes at room temperature. At this stage, do **not** disrupt the hydrogel by tilting or shaking the plate.

Re-suspending the Cells

Procedure

- Harvest the cells to be cultured according to the established protocol.
- Count and re-suspend the cells to the desired density (recommended cell concentration is between 0.5-1 x 10^6 cells/ml); for recommended seeding volumes of culture media for specific well plate sizes see Chapter “5.5 Recommended Volumes for Hydrogel”, page 12).
- Place the well plate in an incubator and change the cover medium every 48 hours. We recommend only changing 50-80% of the cover medium to avoid disturbing the hydrogel or cells.

4.1.3 Harvesting Cells from Hydrogel for Downstream Analyses

Materials:
- Cell recovery solution
- CO_2 incubator
- Centrifuge
- Microtubes

Procedure

- Warm the cell recovery solution to 37 °C.
- Add 100 µL of prewarmed cell recovery solution to each well.
- Gently re-suspend and remove the contents of each well and add to microtubes.
- Incubate at 37 °C for 3-5 minutes.
- Centrifuge microtubes at 300 x *g* for 5 minutes to collect cell pellets for passaging or processing for analysis.

4.2 NexaGel® 3D Cell Culture Protocol

4.2.1 Preparing the Hydrogel and Cell Seeding

Procedure

- Bring the hydrogel to room temperature or warm to 37 °C.
- Harvest the cells to be cultured according to the established protocol. We recommend a cell concentration of 0.5-2 x 10^6 cells/mL.
- Gently mix the hydrogel with cell suspension at 2:1 v/v ratio, e.g., 2 mL of hydrogel plus 1 ml cell suspension.
  - If using cell culture medium with low salt concentration such as RPMI 1640 medium: Consider using 1:1 v/v mixing ratio.
  - To maintain the correct concentration of growth factors, serum, or other media additives: Add these supplements to the hydrogel at 3X concentration to counteract the dilution effect and achieve a final concentration within the hydrogel of 1X.
- Gently pipette up and down 5-10 times to mix thoroughly, taking care to avoid bubble formation.
- Dispense the appropriate volume of hydrogel into each well (for the recommended volumes see Chapter “5.5 Recommended Volumes for Hydrogel”, page 12).
- Gently swirl the well plate to ensure an even covering on the bottom of each well and remove bubbles using ethanol vapor.
- Allow the hydrogel to polymerize for 15 minutes at room temperature. At this stage, do **not** disrupt the hydrogel by tilting or shaking the plate.

Incubating the Well Plate

Materials: CO_2 incubator

Procedure

- Carefully add additional medium to cover the hydrogel (for recommended volumes of cover medium see Chapter “5.5 Recommended Volumes for Hydrogel”, page 12).
- Place the well plate in an incubator and culture at 37 °C with 5% CO_2.
- For long term culture, change the cover medium every 48 hours. We recommend only changing 50-80 % of the cover medium to avoid disturbing the hydrogel or cells.

4.2.2 Harvesting Cells from Hydrogel for Downstream Analyses

Materials:
- P1000 pipette
- CO_2 incubator
- Microtubes

Procedure

- Warm the cell recovery solution to 37 °C.
- Using a P1000 pipette, remove the contents of each well and add them to microtubes.
- Add prewarmed cell recovery solution to each tube at a 5:1 ratio, e.g, per 96 well plate well add 900 µL cell recovery solution per 180 µL hydrogel-cell mixture.
- Gently mix with the P1000 pipette and incubate at 37 °C for 3-5 minutes.
- Centrifuge the microtubes at 300 x *g* for 5 minutes to collect the cell pellets for passaging or processing for analysis.

5 Specifications

5.1 Formulation and Use

Formulation
Xeno-free, polysaccharide-based hydrogel
Use
3D and 2D cell culture
Biocompatible, safe for animal studies

5.2 Hydrogel Properties

Physical state: Liquid
Color: Transparent
pH: Neutral

5.3 Hydrogel Application

Number of uses
2 mL: About 60 uses at 50 µL
10 mL: About 300 uses at 50 µL
Is injectable, e.g. for *in vivo* studies
Harvesting
With NexaGel® Cell Recovery Solution (NGR04-100 | NGR04-500)

5.4 Temperature Conditions and Stability

Temperature conditions
Operation: Room temperature
Storage: +2 °C – +8 °C
Shipping: Ambient temperature
Stability
24 months from date of manufacture (see product label)

5.5 Recommended Volumes for Hydrogel

Well Plate	Hydrogel Volume per Well (µL)
6-well plate	1200
12-well plate	600
24-well plate	300
48-well plate	150
96-well plate	50

6 Sartorius Service

Sartorius Service is at your disposal for queries regarding the product. Please visit the Sartorius website (www.sartorius.com) for information or to contact a local representative.

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

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