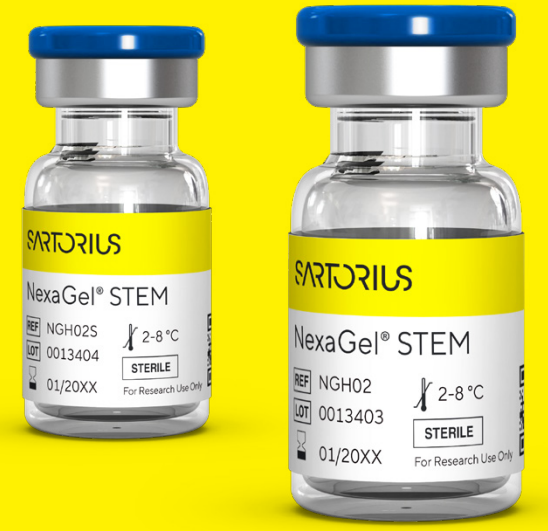
Instructions For Use

Original Instructions For Use

NexaGel® STEM

NGH02 | NGH02S

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 STEM, 10 mL	NGH02
NexaGel® Hydrogel STEM, 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® STEM is a xeno-free (animal origin-free) hydrogel that is intended to enhance the performance of three-dimensional (3D) static suspension cultures and facilitate the scale-up of human pluripotent stem cells (hPSCs).

The product has an optimized formulation that supports the rapid expansion of high-quality 3D stem cell spheroids with pluripotent characteristics. hPSCs can be directly thawed from liquid nitrogen or transferred from 2D matrix-coated culture vessels. The hPSCs can be immediately mixed with the hydrogel solution for static suspension cultures.

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

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 designed for the static suspension culture method. The product allows to simplify and streamline cell harvesting. Microcarriers in large-scale bioreactors are **not** needed.

The static suspension culture method is beneficial for time-sensitive experiments, as it minimizes the need for frequent medium exchanges, and creates a high-throughput platform for modeling various tissues and disease states.

The 3D stem cell spheroids generated can be utilized for further sub-culturing, patterned differentiation, organoid development, or re-establishing 2D culture morphologies.

The product is compatible with most hPSC culture media and tissue culture vessels.

3.2 Culture Cycle for Hydrogel System

3.2.1 NexaGel® STEM 3D Cell Culture Protocol – 96-Well Plate - Passage Every 3 - 4 Days

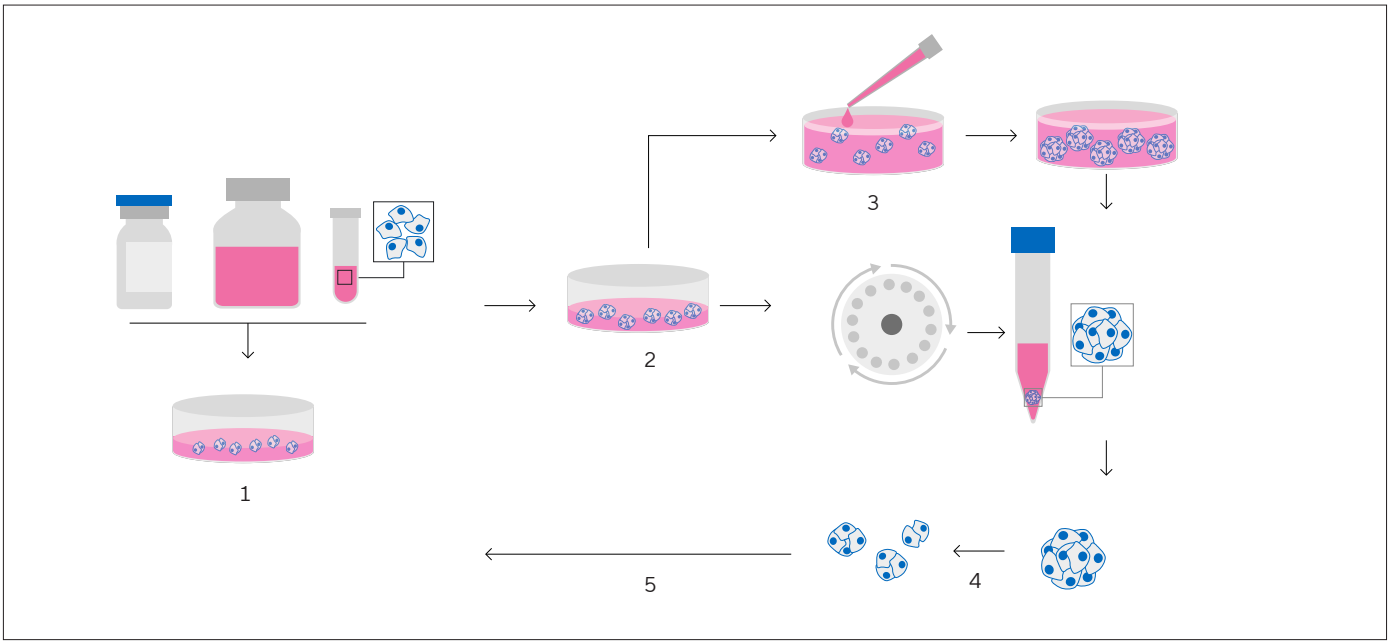

Fig. 1: 3-Day /4-Day Culture Cycle

Pos.	Description
1	Day 0: Seeding cells with the product
2	Day 3 / Day 4

Pos.	Description
3	Collection of cell spheroids via centrifugation or harvesting with cell recovery solution
4	Re-use of cell spheroids for downstream applications
5	Dissociation into smaller clumps to suspend in the product for sub-culture

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 10). 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® STEM 2D Cell Culture Protocol – 96-Well Plate

4.1.1 Seeding

Materials:
- Supplement: ROCK inhibitor Y-27632 (10 mM/mL) or CultureCEPT™ Supplement A56799
- CO_2 incubator

Procedure

- Bring the hydrogel to room temperature or warm to 37 °C.
- Dilute the hydrogel 2:1 with stem cell culture medium.
- Dispense 50 µL into each well, then allow to polymerize for 15 minutes at room temperature.
- Harvest the cells to be cultured according to the established protocol.
- Count and re-suspend the cells to the desired density of 7 x 10^4 cells/mL in stem cell medium with 10 µm/mL Y-27632 or 1X CultureCEPT.
- Seed 100 µL the cell suspension per well for a final density of 7 x 10^3 cells/well.

4.1.2 Harvesting

Materials:
- Cell recovery solution
- 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® STEM 3D Cell Culture Protocol – 96-Well Plate - Passage Every 3 - 4 Days

4.2.1 Seeding

Materials:
- Supplement, e.g., ROCK inhibitor Y-27632 (10 mM/mL) or CultureCEPT™ Supplement A56799
- CO_2 incubator

Procedure

- ▶ Bring the hydrogel to room temperature or warm to 37 °C.
- ▶ Harvest the cells to be cultured according to the established protocol.
- ▶ Prepare the cell suspension in stem cell medium with 10 µm/mL Y-27632 or 1X CultureCEPT.
  - In this example, iPSCs were re-suspended at 1 x 10^6 cells/mL to give a final density once suspended in hydrogel of 5.5 x 10^4 cell/mL.
- ▶ Gently mix the hydrogel with cell suspension at 2:1 v/v ratio; 2 mL of the hydrogel plus 1 mL cell suspension.
- ▶ Add the stem cell medium with 10 µm/mL Y-27632 or 1X CultureCEPT to the cell-hydrogel mixture at 5:1 v/v ratio according to recommended volumes (see Chapter “5.5 Recommended Volumes for 3D Cell Culture Protocol”, page 11).
- ▶ Gently pipette up and down to mix homogeneously.
- ▶ Add the mixture to the culture vessel wells and incubate at 37 °C with 5% CO_2.

4.2.2 Harvesting

Materials:
- Cell recovery solution
- P1000 pipette
- Centrifuge
- Microtubes

Procedure

- ▶ Warm the cell recovery solution to 37 °C.
- ▶ Using the pipette, remove the contents of each well and add to micro-tubes.
- ▶ Add the prewarmed cell recovery solution to each tube at a 5:1 ratio; per 96-well plate well add 900 µL cell recovery solution per 180 µL hydro-gel-cell mixture.
- ▶ Gently mix with the 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 static suspension culture for hPSCs
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: Suitable for 15-30 mL suspension culture
10 mL: Suitable for 90-180 mL suspension culture
Is injectable, e.g. for *in vivo* studies
Cell 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 3D Cell Culture Protocol

Solution	Hydrogel Volume per Well (µL)
Hydrogel	20
Cell suspension	10
Stem cell culture medium	150
Total volume per well	108

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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