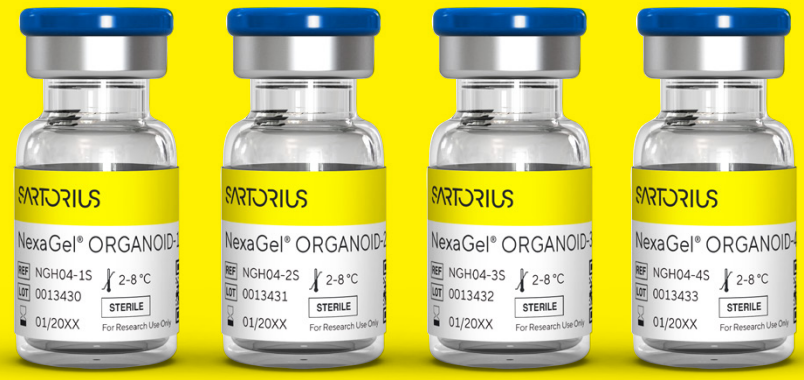
Instructions For Use

Original Instructions For Use

NexaGel® ORGANOID

NGH04-K | NGH04-1 | NGH04-2 | NGH04-3 | NGH04-4

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® ORGANOID Discovery Kit	NGH04-K
NexaGel® ORGANOID-1	NGH04-1
NexaGel® ORGANOID-2	NGH04-2
NexaGel® ORGANOID-3	NGH04-3
NexaGel® ORGANOID-4	NGH04-4

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® ORGANOID is a xeno-free (animal origin-free) hydrogel that is intended to support the cultivation of patient-derived organoids, organoids from pluripotent stem cells (PSCs), co-cultures, and PDX models.

The product is suitable for 3D cell culture and 2D hydrogel coating applications. The product can be used alongside NexaGel® STEM, a hydrogel system designed for 3D static suspension cultures and the expansion of human pluripotent stem cells.

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 with varying bio-functional ligands, mechanical properties, and biodegradability to cater to diverse organoid culture needs. The product offers a well-defined 3D microenvironment, advancing the field of personalized medicine.

Organoids grown can be easily retrieved using a recovery solution.

3.2 Culture Methods

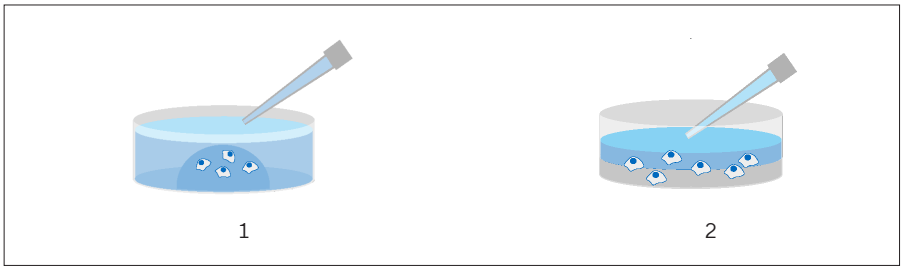

Fig. 1: Overview of culture methods

Pos.	Name
1	3D Dome
2	3D Cell Culture Encapsulation

3.3 Hydrogel Preparation Workflow

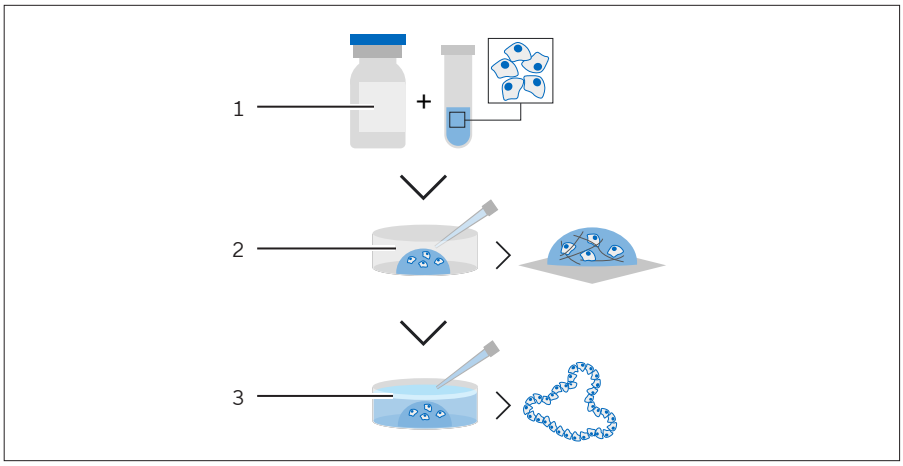

Fig. 2: 3D dome method (example)

Pos.	Description
1	Mixing of hydrogel and cell culture medium
2	Addition as a dome & incubation
3	Addition of medium & incubation

3.4 Guidance for Culturing Organoids in NexaGel®

Due to the large variety of organoid types, origins, and culture conditions, transferring organoid culture to the NexaGel® product may require extensive optimization. Some guidelines for this process are suggested here.

3.4.1 Growth Factor Control

NexaGel® is a synthetic hydrogel, free of any extraneous growth factors or nutrients that would be present in an animal-derived extracellular matrix (ECM). This provides users with full control over the growth factor content of the culture, but may result in slower growth of organoids or require additional supplementation to support organoid expansion compared to animal-derived matrices.

As a starting point, we recommend increasing the concentration of critical medium supplements to 3X the desired final concentration when preparing the hydrogel. The suggested mixing ratio is 2:1 of product and medium, maintaining the final concentration of growth factors/supplements in the hydrogel at 1X.

Please note that this supplement increase is only recommended for the initial hydrogel preparation stage, and for any medium or cell suspensions to be added on top of the hydrogel the supplements can be maintained at 1X concentration.

Depending on the composition of the user's media, further formulation adjustments may be required.

3.4.2 Hydrogel Concentration

In addition to media supplementation, the hydrogel concentration may require optimization. For softer/less rigid hydrogels, dilute the hydrogel solution with DI water, e.g., in a 1:1 ratio, before mixing the hydrogel with cell medium/cell suspension. The ideal ratio will depend on cell type and require multiple rounds of optimization by the user.

3.4.3 Hydrogel Type

The NexaGel® ORGANOID hydrogel is offered in 4 different varieties. Each organoid hydrogel is formulated with various mechanical strengths, functional ligands, and degradability to suit a range of tissues and conditions. Users will need to determine which hydrogel is optimal for their organoid type. A Discovery Kit, with all 4 varieties, is available to aid in identifying a suitable hydrogel.

For hydrogel suitability for various organoid types see Chapter “5.2.1 Hydrogel Suitability for Organoid Types”, page 11.

3.5 Cell Harvesting

Harvesting of cells for downstream analysis is possible by using a recovery solution (see Chapter “5.3 Hydrogel Application”, page 12). With the 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 3D Dome Protocol

NexaGel® ORGANOID-1 is used as an example in this protocol, substitute it with alternative NexaGel® products as appropriate.

We recommend to use non-tissue culture treated plates for dome formation. This will prevent the dome from detaching from the surface of the well.

We recommend to prepare media containing 3X critical supplement/ growth factors for mixing with the hydrogel and cells, to produce a final concentration within the NexaGel of 1X.

Bubbles can be avoided by the following measures:

- Using reverse pipetting technique
- Warming the hydrogel to 37 °C before use
- Using IMS vapor to remove bubbles. To do this, use a wash bottle containing a small amount of IMS and with the inner straw removed to gently blow vapor over the hydrogel after pipetting into the tissue culture plate.

Procedure

- ▶ Warm the hydrogel to room temperature.
- ▶ Prepare the cell/organoid suspension in media containing 3X growth factors or supplement. Cell concentration should also be 3X the desired final concentration.
- ▶ Add 2 parts hydrogel, e.g., 200 µL to 1 part cell suspension, e.g., 100 µL.
- ▶ Gently pipette up and down 5-10 times to mix thoroughly. Keep the hydrogel and the cell suspension at 2:1 v/v mixing ratio.
- ▶ Add 30 µL of the hydrogel-cell mixture to the center of each well of a 24-well plate (non-tissue culture treated). Dispense the droplets as soon as possible after adding the hydrogel solution to the cell suspension, as this will initiate soft gel formation and may interfere with pipetting.
- ▶ Let the hydrogel stabilize at room temperature for 15-20 min for soft gel formation.
- ▶ Carefully add 1 mL of culture medium to each well of the 24-well plate without disturbing the hydrogel.

Incubating the Well Plate

Procedure

- ▶ Place the well plate in an incubator and change the medium according to experimental time frame.
- ▶ We recommend only changing 50-80 % of the cover medium when feeding organoids to avoid disturbing the hydrogel.
- ▶ If a different medium is required during organoid culture: Change 100 % of the medium.

4.2 3D Cell Encapsulation Protocol

NexaGel® ORGANOID-1 is used as an example in this protocol, substitute it with alternative NexaGel® products as appropriate.

We recommend to prepare media containing 3X critical supplement/ growth factors for mixing with the hydrogel and cells, to produce a final concentration within the NexaGel of 1X.

Bubbles can be avoided by the following measures:

- Using reverse pipetting technique
- Warming the hydrogel to 37 °C before use
- Using IMS vapor to remove bubbles. To do this, use a wash bottle containing a small amount of IMS and with the inner straw removed to gently blow vapor over the hydrogel after pipetting into the tissue culture plate.

Procedure

- ▶ Warm the hydrogel to room temperature.
- ▶ Prepare the cell/organoid suspension in media containing 3X growth factors or supplement. Cell concentration should also be 3X the desired final concentration.
- ▶ Add 2 parts hydrogel, e.g., 1000 µL to 1 part cell suspension, e.g., 500 µL.
- ▶ Gently pipette up and down 5-10 times to mix thoroughly. Keep the hydrogel and the cell suspension at 2:1 v/v mixing ratio.
- ▶ Transfer the appropriate volume of hydrogel mixture to a well plate (for recommended volumes see Chapter “5.5 Recommended Volumes for 3D Cell Encapsulation Protocol”, page 12).
- ▶ Dispense the mixture as soon as possible after adding the hydrogel solution to the cell suspension, as this will initiate soft gel formation and may interfere with pipetting.
- ▶ Gently tilt/swirl the well plate to ensure there is an even coverage on the bottom of each well.

Incubating the Well Plate

Procedure

- ▶ Incubate the plate for 15-20 min at room temperature to allow for soft gel formation. At this stage, do **not** disrupt the hydrogel by tilting or shaking the plate.
- ▶ Carefully cover the hydrogel with additional medium (for recommended volumes see Chapter “5.5 Recommended Volumes for 3D Cell Encapsulation Protocol”, page 12).
- ▶ Place the well plate in an incubator and change the medium according to experimental time frame.
- ▶ We recommend only changing 50-80 % of the cover medium when feeding organoids to avoid disturbing the hydrogel.
- ▶ If a different medium is required during organoid culture: Change 100 % of the medium.

5 Specifications

5.1 Contents, Formulation and Use

	Unit	Value
Contents		
NexaGel® ORGANOID Discovery Kit, consisting of 1 piece of NexaGel® ORGANOID-1 | ORGANOID-2 | ORGANOID-3 | ORGANOID-4, each	mL	2
NexaGel® ORGANOID-1	mL	10
NexaGel® ORGANOID-2	mL	10
NexaGel® ORGANOID-3	mL	10
NexaGel® ORGANOID-4	mL	10
Formulation		
Xeno-free, polysaccharide-based hydrogel		
Use		
Organoid culture		
Injectable hydrogel for in vivo studies and laboratory automation		

5.2 Hydrogel Properties

Physical state: Liquid
Color: Transparent
pH: Neutral

5.2.1 Hydrogel Suitability for Organoid Types

Hydrogel Type	**Organoid Type**			
	Gastric	Lung	Brain	Cancer
NexaGel® ORGANOID-1	X	X	-	-
NexaGel® ORGANOID-2	X	-	X	-
NexaGel® ORGANOID-3*	-	X	X	-
NexaGel® ORGANOID-4	-	-	-	X

* Most ‘generic’ hydrogel that may be suitable for growth of most organoid types and a good starting point for experiments.

5.3 Hydrogel Application

Number of uses, dilution ratio
2 mL: 80 uses, at 25 µL per dome | 40 uses, at 50 µL per dome
10 mL: 400 uses, at 25 µL per dome | 200 uses, at 50 µL per dome
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 3D Cell Encapsulation Protocol

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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