Instructions For Use

Original Instructions For Use

NexaGel® Cell Recovery Solution

NGR04-100 | NGR04-500

Solution for harvesting organoids or cells

1000143934

SARTORIUS

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® Cell Recovery Solution, 100 mL	NGR04-100
NexaGel® Cell Recovery Solution, 500 mL	NGR04-500

1.2 Related Documents

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

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® Cell Recovery Solution is a non-enzymatic solution that is intended for the harvesting of organoids or 3D cells cultured with NexaGel® hydrogel or animal-based ECM (extracellular matrices).

The product can be mixed with the cells, either before or after the fixation and staining of the hydrogel mixture, or used with 2D ECM-coated plates. The recovered cells can be sub-cultured in both 2D and 3D formats.

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 solution is a ready to use product that allows a rapid and efficient harvesting of organoids or 3D cells from NexaGel® hydrogel mixtures or animal-based ECM. The product ensures a high recovery rate and cell viability, making it perfect for passaging, cryopreservation, or further biochemical analysis.

3.2 Organoid and Cell Recovery Workflow

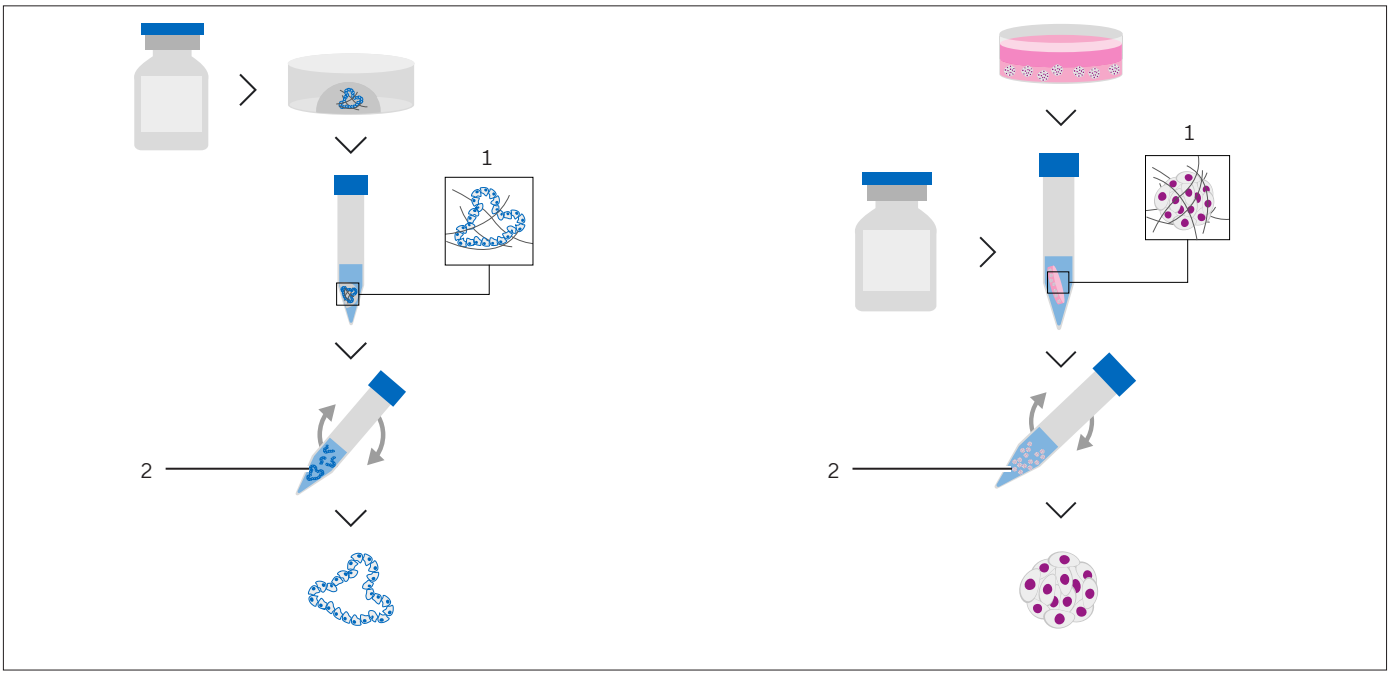

Fig. 1: Recovery from animal-based ECM | recovery from hydrogel

Pos.	Description
1	Mix of product and cells, and transfer to a tube
2	Rocking and centrifugation

4 Protocol

4.1 Cell/Organoid Recovery from Animal-based ECM

4.1.1 Preparing the Hydrogel

Procedure

- ▶ Chill the hydrogel to 4 °C before use for optimal performance.

4.1.2 Recovering Cells/Organoids from ECM

Materials: Centrifuge tube, 15 mL

Procedure

- ▶ Remove the medium from the animal-based ECM culture plate.
- ▶ Add the pre-chilled hydrogel to each well containing animal-derived matrix (for recommended volumes see Chapter "5.4 Recommended Volumes for Hydrogel", page 10).
- ▶ Pipette gently up and down to mechanically break down the hydrogel.
- ▶ Transfer the hydrogel and solution together to the centrifuge tube.
- ▶ If residue is visible in the well:
  - ▶ Add additional product to wash the plate.
  - ▶ Ensure all material is harvested and add it to the centrifuge tube.

4.1.3 Performing ECM Dissociation

Procedure

- ▶ Mix the harvested material in the centrifuge tube:
  - ▶ Option 1: Gently pipette up and down 5-10 times.
  - ▶ Option 2: Rock the centrifuge tube for 2-5 minutes.
- ▶ Chill the centrifuge tube on ice or place in refrigerator for 2-5 minutes for ECM dissociation.
- ▶ Centrifuge at 100 x *g* for 3-5 minutes at 4 °C.
- ▶ Remove the supernatant. The cells should be ready for subculture or storage.

4.1.4 Re-suspending the Cells to Completely Remove the ECM (Optional)

Because sources of animal-based ECM have varying protein concentrations, it may require to re-suspend the cells in additional product to completely remove the ECM. This will break the organoids into smaller fragments.

Procedure

- ▶ Re-suspend the cells in additional product.
- ▶ Perform the ECM dissociation step (see Chapter “4.1.3 Performing ECM Dissociation”, page 7).

4.2 Cell/Organoid Recovery from Hydrogel

4.2.1 Preparing the Hydrogel

Maintaining the product at 37 °C is important throughout the whole process to facilitate ionic release from the solid hydrogel and allow it to form a soft hydrogel.

Mechanical force such as rocking or pipetting to mix the hydrogel with the recovery solution also accelerates the transition to a liquid state.

Adding the product to a level of 10x that of the hydrogel volume will also maintain the hydrogel in a liquid state.

Procedure

- ▶ Warm the hydrogel to 37 °C.

4.2.2 Recovering Cells/Organoids from Hydrogel

Materials:
- DPBS
- Conical tube, 15 mL

Procedure

- ▶ Take the culture plate out of the incubator and remove the medium covering the top of the hydrogel.
- ▶ Wash the hydrogel two times with DPBS.
- ▶ Add warm product to the well plate (for recommended volumes see Chapter “5.4 Recommended Volumes for Hydrogel”, page 10).
- ▶ Use a pipette to gently break the hydrogel into small pieces by gently pipetting up and down. This step can accelerate the hydrogel dissolution process.

4.2.3 Performing Hydrogel Dissociation

Procedure

- ▶ Add 5 mL warm product to the conical tube and transfer the hydrogel to the tube.
- ▶ If residue is visible in the well:
  - ▶ Add additional product to wash the plate.
  - ▶ Ensure all material is harvested and add it to the tube.
- ▶ Repeat the following step 2-3 times. Depending on cell type, gel type, and gel concentration, this step may require optimiation:
  - ▶ Use a pipette to gently mix the solution 2-5 times.
  - ▶ Put the tube into a water bath at 37 °C for 2- 3 min.
- ▶ Centrifuge at 100 x *g* for 3-5 minutes at room temperature to collect the cell pellet. The speed and time can be adjusted depending on cell type.
- ▶ If there is still some hydrogel on top of the cell pellet: Re-suspend the cells (see Chapter 4.2.4, page 9).

4.2.4 Re-suspending the Cells to Completely Remove the Hydrogel (Optional)

It may require to re-suspend the cells in additional product to completely remove the hydrogel. This will break the organoids into smaller fragments.

Procedure

- ▶ Re-suspend the cells in additional product.
- ▶ Perform the hydrogel dissociation step (see Chapter “4.2.3 Performing Hydrogel Dissociation”, page 9).

4.3 Using Alternative Protocols

Procedure

- ▶ If you need to adapt the protocol to your applications: Please contact Sartorius for further support.

5 Specifications

5.1 Formulation and Use

Formulation
Non-enzymatic for stable, safe and efficient cell recovery
Use
Suitable for the recovery of intact organoids as well as 3D cells
Allows complete and fast ECM dissociation of animal-based ECM, for intact organoids/cells and high recovery rate for organoid expansion
Recovered cells can be sub-cultured in both 3D and 2D
Supports cell recovery from 2D ECM coating plates

5.2 Hydrogel Properties

Physical state: Liquid
Color: Transparent
pH: Neutral

5.3 Temperature Conditions and Stability

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

5.4 Recommended Volumes for Hydrogel

Well Plate	**Hydrogel Volume per Well (µL)**
6-well plate	2000
12-well plate	1000
24-well plate	500
48-well plate	250
96-well plate	100

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.

Sartorius BioAnalytical Instruments Inc.
565 Johnson Avenue
Bohemia, New York 11716

Phone: +16312544249
www.sartorius.com

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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Sartorius BioAnalytical Instruments Inc.
565 Johnson Avenue
Bohemia, New York 11716

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