AXOL

Discovery Stems From Here

Hydrogel Protocol

Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival
ax494319	Hydrogel 5 ml syringe	5 mL	N/A	Follow the protocol
ax494319	Hydrogel 10 ml syringe	10 mL	N/A	Follow the protocol
ax494341	Cell Recovery Enzyme 2.5 ml	2.5 mL	N/A	Follow the protocol

Hydrogel

Axol's biocompatible hydrogel is extracted from birch (Betula sp.), sourced from sustainable and responsibly managed forests. The Hydrogel consists of micro and nanosized cellulose fibrils, the length of fibrils may be several micrometers while the diameter is on the nanometer scale (4-100 nm). The product is supplied ready to use in a syringe format at a working concentration of 1.5% cellulose and 98.5% ultra-pure water. It is not a concentrate. The hydrogel is not auto fluorescent, is pH neutral and is compatible with automated liquid handling systems, high content imagers and standard microscopes set ups.

The hydrogel supports culture of a variety of cells including iPS, ES, cancer, retinal, neural cells as well as patient derived cells and biopsies and used for 3D organoids and spheroid culture, supporting cell growth and differentiation with consistent results.
Tested applications range from 3D cell culture and organ-on-a-chip models, through to drug release studies and 3D printing.

Eliminating the need for temperature control on the bench, Axol's hydrogel is stored, shipped and suitable for use at room temperature, reducing complexity and cost. There is no batch to batch variability.

***NB**: The product has been autoclaved at a temperature of 120°C. The product should be handled in accordance with good industrial hygiene and safety practices. Use protective gloves and clothes to avoid skin exposure. If exposed, wash the exposed skin with water. Use protective laboratory eyewear to avoid contact with the eyes. In its wet state the product does not form dust. However, if dried, avoid breathing the dust. Dust filters are recommended.*

Storage Instructions

The product should be stored in the dark 5-22°C for optimum performance. Once opened it is recommended that the product is stored undiluted at 2-8°C for a maximum of 3 months. If the product has been diluted, e.g. with culture media, then it should be stored at 2-8°C for a maximum of 7 days. If the media contains an unstable component, then storage time will be restricted to the shelf life of the given component. Do not store the product below 0°C as freezing will result in destabilization of the product, rendering it unusable.

General Recommendations

- Use a low retention pipette tips to avoid Hydrogel sticking to the tip.
- Aspirating and dispensing Hydrogel should be performed slowly to avoid air bubbles and to ensure an accurate volume.
- For an exact amount of undiluted Hydrogel, the product can be weighed before dilution.
- Electric dispensing pipettes can be used for mixing to reduce user variability.
- A multi-stepper pipette is recommended for repeated dispensing of Hydrogel into the well plates.
- If Hydrogel has accumulated on the walls of the vial or water droplets are present, then the vial can be centrifuged briefly to concentrate the product at the bottom for easier recovery.
- Microplates containing Hydrogel should be handled with care. Avoid shaking the plate when moving it between locations.
- When culturing adherent cells, the use of low-attachment microplates or pre-coating with polyHEMA is recommended to prevent cells attaching to the bottom of the wells.
- When changing the medium, Extra care should be taken not to disturb the top of the gel. If loss of Hydrogel occurs when changing medium, then it is recommended to exchange only half the amount of media at one time.

Recommended Procedure for Diluting and Mixing Hydrogel

The viscosity can be adjusted by diluting the product to a less viscous state. Dilution can be made with cell culture media, PBS, or ultra-pure water. Hydrogel concentrations of 0.2-1.0% are typically used for cell culture applications. The optimal concentration will depend on the cell type being used. We recommend a concentration of 0.5% of Hydrogel, per cell seeding density of 1000 cells/µl.

Example dilution protocols for 3D cell culture experiments

Working concentration required = 0.5 %. Final volume = 1 ml.

- Pipette 567 µl culture medium into a test tube.
- Add 333 µl Hydrogel and mix by pipetting up and down until the solution is homogenous by visual inspection.
- Add 100 µl cell suspension to the diluted Hydrogel slowly and stir carefully using the pipette tip to evenly disperse the cells.
- Hydrogel is now ready for use at a working concentration of 0.5 %.

***NB**: Pre-diluted Hydrogel without cells can be stored for 7 days at 2-8°C if no unstable components are present in the media.*

Dilution Table

Volume of Hydrogel, diluent and cell suspension required for the preparation of 1 ml of diluted Hydrogel for a variety of final working concentrations.

Final Hydrogel Concentration	Total Volume	Volume of Hydrogel Stock Solution 1.5%	Diluent	Cell Suspension
1%	1 ml	667 µl	233 µl	100 µl
0.9%	1 ml	600 µl	300 µl	100 µl
0.8%	1 ml	533 µl	367 µl	100 µl
0.7%	1 ml	467 µl	433 µl	100 µl
0.6%	1 ml	400 µl	500 µl	100 µl
0.5%	1 ml	333 µl	567 µl	100 µl

Cell Recovery Enzyme

Axol's Cell Recovery Enzyme is a cellulase enzyme mixture in aqueous sodium acetate buffer solution, pH 5. The Enzyme is exclusively designed for the digestion of the bio-friendly Axol' Hydrogel enabling recovery of cells, spheroids, organoids or tissue samples intact.

Axol's Cell Recovery Enzyme is applied using a very simple process. The enzyme is added before incubation where cells or organoids are recovered which eliminates the damage caused by repeated pipetting steps. Cells once recovered can be replated or used in downstream DNA/RNA analysis without interference from the hydrogel and the enzyme, which are both from a plant source. Axol's Cell Recovery Enzyme and Hydrogels do not impact cell survival or function.

The enzyme is mixed with the sample and incubated at 37°C until the Hydrogel has been fully reduced to glucose. Enzymatic removal allows cells to be recovered from the matrix efficiently and retain their 3D structure. The amount of Cell Recovery Enzyme required for cell recovery is dependent on the amount of Hydrogel (cellulose) present in the sample. It is recommended that the enzyme be used at a working concentration of 300 µg/mg. An equal volume of working concentration of Cell Recovery Enzyme to Hydrogel/cell matrix volume present in the sample should be used, e.g. if 100 µl of Hydrogel/cell matrix is present in the sample then 100 µl of Cell Recovery Enzyme (300 µg/mg) should be added to the sample.

NB: Hazard Statements: The Cell Recovery Enzyme may produce an allergic reaction.

Storage Instructions

The unopened product should be stored in the dark at 5-22°C for optimum performance. Once opened it is recommended that the product is stored at 2-8°C for a maximum of 6 months. If the product has been diluted, e.g. with culture media, then it should be used immediately. Any leftover diluted enzyme should be discarded.

Procedure for Removing Hydrogel by Enzymatic Degradation

- Calculate the amount of Hydrogel present in the sample well using the following equation, where 100 µl of 1% Hydrogel = 1 mg.
  Sample well volume (µl) x % Hydrogel concentration / 100 = mg Hydrogel / sample well.

- Calculate the amount of enzyme needed to degrade the Hydrogel in the sample well using the following equation, where 300µg Enzyme is required to degrade 1mg of Hydrogel.
  Amount (mg) Hydrogel / sample well x 300 = µg Cell Recovery Enzyme.

- Calculate the volume of Cell Recovery Enzyme stock solution needed using the following equation: Cell Recovery Enzyme amount (µg) / Cell Recovery Enzyme stock concentration (10 µg/µl) = Volume (µl) of Cell Recovery Enzyme stock solution.

- Prepare the working concentration of Cell Recovery Enzyme by diluting the stock solution with culture media as follows: Sample well volume (µl) - Volume (µl) of Cell Recovery Enzyme stock solution (10 mg/ml) = Volume of culture media for dilution.

- Pipette the diluted Cell Recovery Enzyme onto the top of the sample in the microplate. Incubate the plate at 37°C for a minimum of 8 hours until the hydrogel has fully degraded. Recover the cells from the well using standard techniques.

Example Experimental Procedure

Materials: 80 µl of 0.9% Hydrogel/cell mix per well in a 96-well microplate, Cell Recovery Enzyme (10 mg/ml), Cell culture medium.

- Amount of Hydrogel present in the sample:
  80 µl x 0.9 / 100 = 0.72 mg Hydrogel / sample well
- Amount of Cell Recovery Enzyme required to degrade Hydrogel in sample:
  0.72 mg x 300 µg/mg = 216 µg Cell Recovery Enzyme
- Volume of Cell Recovery Enzyme stock solution needed:
  216 µl / 10 µg/µl = 21.6 µl Cell Recovery Enzyme stock solution.
- Prepare the working concentration of Cell Recovery Enzyme by diluting the stock solution (10 mg/ml) with culture media:
  80 l - 21.6 µl = 58.4 µl culture media for dilution.
- Pipette the diluted Cell Recovery Enzyme onto the top of the sample in the microplate. Incubate the plate at 37°C until the hydrogel has fully degraded. Recover the cells from the well using standard techniques.

Cell Recovery Enzyme Dilution Table

Volume of Cell Recovery Enzyme and cell culture medium required for the preparation of 100 µl of Cell Recovery Enzyme working solution (300 µg/mg) for the degradation of 100 µl of Hydrogel.

Hydrogel Conc. In 100 µl of Sample	Amount of Cell Recovery Enzyme Needed	Volume of Cell Recovery Enzyme Stock Solution (10 mg/ml)	Volume of Cell Culture Medium
1%	300 µg	30 µl	70 µl
0.9%	270 µg	27 µl	73 µl
0.8%	240 µg	24 µl	76 µl
0.7%	210 µg	21 µl	79 µl
0.6%	180 µg	18 µl	82 µl
0.5%	150 µg	15 µl	85 µl
0.4%	120 µg	12 µl	88 µl
0.3%	90 µg	9 µl	91 µl

Usage Statement

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans.

Notes

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
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Or
call +44 (0) 1223 751051