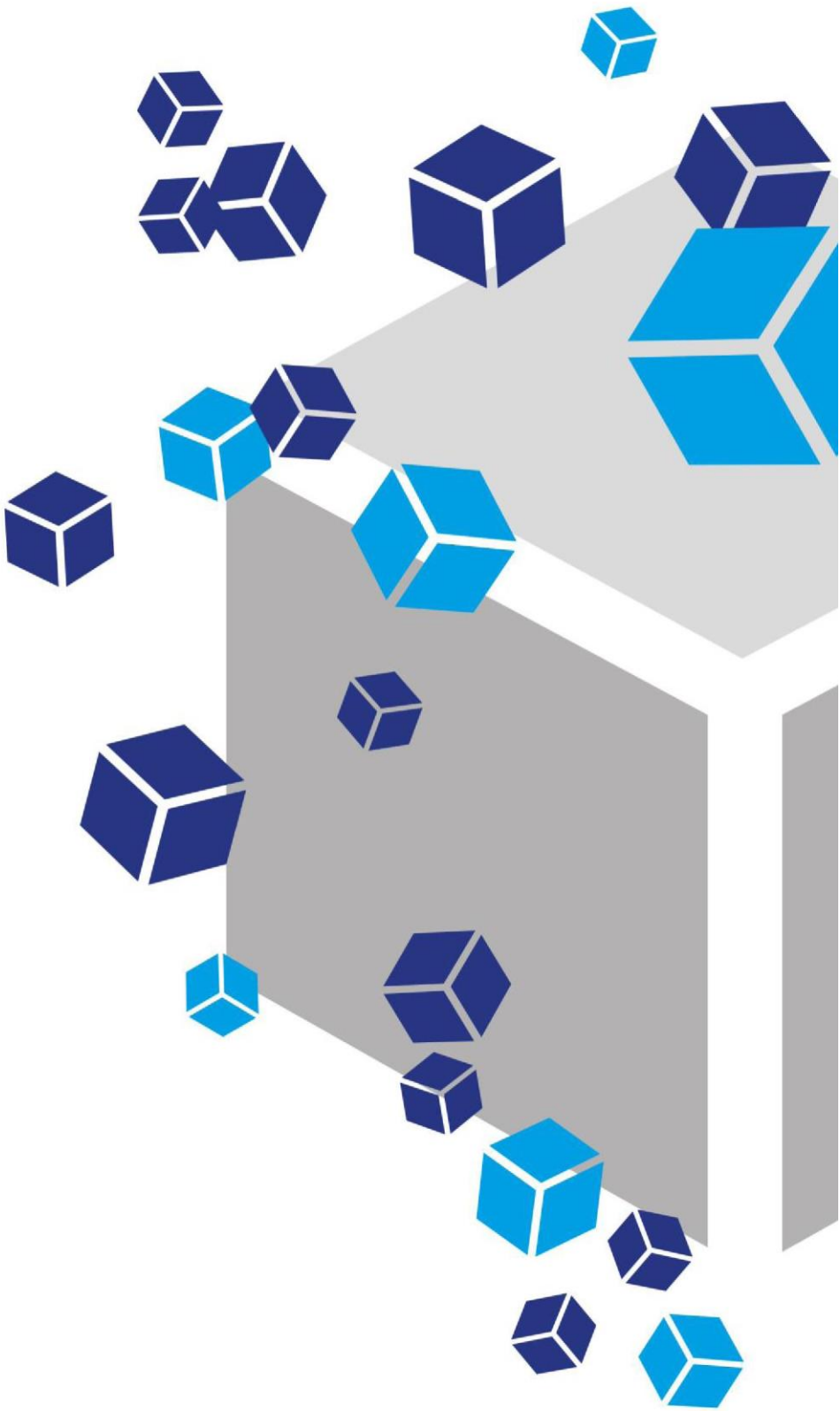
CELL guidance systems

User Guide



# PODS®

## Sustained Release Growth Factors

Version 6.1

# Contents

# PODS®
# Formulated Sustained Release Growth Factors

## Introduction to PODS®

POlyhedrin Delivery System (PODS®) is a formulation that provides an effective solution to the inherent instability of proteins. PODS® technology exploits the natural properties of the polyhedrin protein which forms in-cell crystals (within a cell). PODS® are co-crystals formed when a tagged protein of interest (the bioactive protein) is co-expressed with the polyhedrin protein. The polyhedrin protein forms regular, cubic crystals, around 0.5 - 7 microns in size, within which the bioactive protein specifically binds via a short protein immobilization tag. PODS® degrade under the activity of proteases to provide a slow-release depot formulation for the bioactive protein.

## Storage

**Upon receipt, store at 4°C.**

Lyophilized PODS® are stable for at least a year at 4°C.

### Reconstitution

PODS® may be reconstituted at 1 µg/ml in water. Alternatively, 20% glucose has a buoyant density closer to PODS® crystals and can be useful to reduce the rate of sedimentation when aliquoting. PODS® crystals are highly stable when stored in aqueous solution (pH range 6-8) at 4°C and have been shown to maintain >70% stability for at least 6 months.

## Physical characteristics

### Size and shape

PODS® crystals are cubic and typically 0.5-7 microns in size with a modal size of 0.9-1 microns. During the manufacturing process, some PODS® may be chipped resulting in smaller crystal fragments.

## Buoyant density

PODS® crystals are slightly heavier than water and will slowly settle on the surface of a culture vessel. Care should be taken when aliquoting, since PODS® crystals will sink to the bottom of a tube within a few minutes. The majority of PODS® crystals will remain in suspension for up to 60 min in a 20% glucose solution (or a solution of similar density). Physical factors affecting stability PODS® are highly stable when stored in solutions between pH 6-8. PODS® are also stable at 37°C for extended periods of time (> 10 days). Above pH 10, PODS® lose their stability and can dissolve within a few hours, particularly at elevated temperatures. Acids <pH5 can infiltrate PODS and damage their cargo protein.

## Elution characteristics

Since PODS® are protein structures, they are degraded in solutions that contain proteases. **PODS® do not readily degrade or release the active protein in simple aqueous buffers.** Proteases may be derived from components of the solution (e.g. serum) or secreted by cells. Consequently, the culture system affects the amount of growth factor available in solution. In contrast to gelencapsulated proteins (made using hydrogels such as PLGA), PODS® crystals do not produce burst release. Example elution characteristics are shown below for PODS® LIF in cell culture. In this system, peak availability of soluble LIF released from PODS® occurs at day 2, then gradually diminishes.

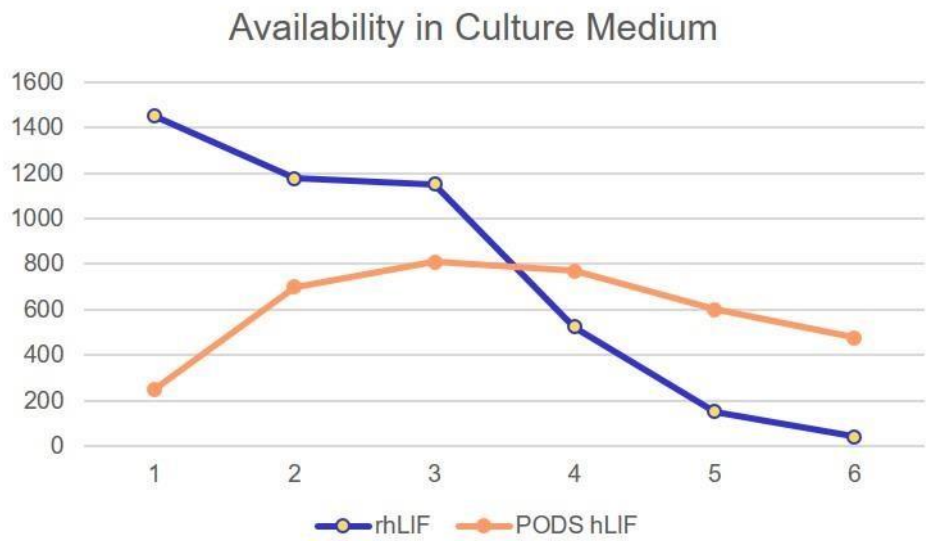



Figure 1. The concentration (pg/ml) of a recombinant growth factor or a PODS® growth factor in solution was measured using an ELISA assay over a period of 6 days. At the start of the experiment, on day 1 a conventional recombinant growth factor (rhLIF) was maximal but was completely depleted by day 6. In contrast >50% of peak levels were still present for PODS® LIF proteins on day 6. (Nishishta et al 2011).

## Modifying the release profile

Release of active proteins into cell culture occurs over a period of 4-8 weeks. This release period can be extended to several months if the PODS® are combined with a secondary hydrogel scaffold, such as collagen or PeptiGel. We have successfully incorporated PODS® into hydrogel microspheres.

## Other characteristics

PODS® adhere to most plastics. For aliquoting, we recommend using low adherence plastics, such as low protein binding centrifuge tubes made by Nunc.

## Resuspending PODS®

PODS contain a bioactive cargo protein contained within a polyhedrin crystal lattice. The majority of protein contained within in PODS crystals is polyhedrin. The bioactive cargo protein typically makes up from 0.5 – 5% of the combined amount.

PODS proteins are provided as dried down aliquots in standard pack sizes of 25 µg, 250 µg and 1 mg. This pack size refers to the amount of the bioactive cargo protein contained within PODS. The amount of combined (cargo + polyhedrin) protein supplied will depend on the cargo protein : polyhedrin protein ratio.

For example, a 25 µg pack size with a 2% cargo loading ratio will contain (100% / 2%) x 25 = 1.25 mg of combined protein.

Resuspend PODS in PBS or serum-free media to provide a fixed concentration of the cargo protein. For example, resuspending a 25 ug pack size in 250 ul will provide a stock concentration of 100 ng/ml

## How much PODS® cargo should be used?

Under the action of proteases, which degrade the polyhedrin scaffold protein, PODS provide sustained release of the cargo protein. Any cargo growth factor molecule contained within PODS is not immediately available to cells and not bioavailable whilst inside the PODS. Once released, growth factors become bioavailable to bind cells receptors. The concentration to which a growth factor accumulates in cell culture media (or in-vivo environment) will depend on the amount of cargo (contained in PODS) added, the rate of cargo release, and the subsequent rate of degradation of the released cargo protein.

As a rule of thumb, in the presence of 10% serum, peak levels of bioavailable growth factors released from PODS are reached within 24-48 hours. Typically, at this point 20% of the growth factor cargo initially contained within the PODS is present in a soluble form and available to bind cells. For example, if PODS containing 100 ng of cargo are added to 10 ml of cell culture media containing 10% serum, it can be expected that 20 ng will be released after 24 hours to give a concentration of available growth factor of 2 ng/ml.

The concentration that you need for a particular application will likely be lower than the equivalent conventional growth factor. This is because PODS are better at maintaining minimum growth factor concentrations. Pre-incubating PODS with serum or other sources of proteases for 24 hours prior to culture will ensure that available growth factor is immediately present. Conventional growth factor may also be spiked in to provide bioavailable growth factor for the first few hours. Ultimately, the amount of PODS growth factor that is optimal for a particular experiment should be optimized empirically.

## How often should media and PODS® crystals be replaced?

The required frequency of media change depends on (a) the rate at which nutrients are exhausted or degraded and (b) the rate at which toxic metabolites accumulate. In most cell culture systems, stability of growth factors is an overriding issue which drives media replenishment. Unformulated growth factors are typically used in excess to compensate for their instability. High concentrations can over-stimulate cells leading to high levels of metabolism. This can accelerate media depletion

and metabolite accumulation. When using PODS, changing half the media is likely to be more effective than complete media changes as this allows retention of released growth factors. As with any protocol, optimization is required.

### Equivalence to standard growth factors

The stability of standard recombinant growth factors varies significantly. The most labile, such as FGF-1, have an in-vivo serum half-life measured in minutes (Zakrzewska et al) limiting utility. The stability of PODS® growth factors is much longer, but once released, cargo protein stability will be the same as standard recombinant counterparts.

### Refractivity

PODS® are crystals and therefore refract light. Large numbers of PODS® may interfere with the use of imaging techniques such as phase-contrast microscopy and will also interfere with measurements based on absorbance. When using a colorimetric assay, the PODS® crystals should be excluded from the assay chamber. This may be achieved by removing the cell culture media into a separate plate for measurement. Alternatively, adding alkaline buffer to dissolve the PODS® crystals may be effective (see the section “Western Blotting”).

### Physical impact on cell behavior

Physical features on a culture surface may impact the behavior of certain cells. If this is a concern, PODS® crystals may be incorporated into a hydrogel surface coating. Matrigen™ Softwell plates (flat, 2D hydrogels of defined elasticity) containing PODS® are available as a custom order from Cell Guidance Systems. Many tissue culture plasticware suppliers including Corning, Falcon, Nunc and Sarstedt offer sterile permeable hanging inserts for tissue culture plates which separate the components of culture.

## Biological characteristics

### Post-translational modifications

PODS® are manufactured using insect cells. Proteins made in insect cells contain most of the posttranslational modifications that are found in mammalian cells.

### Immunogenicity

The polyhedrin protein has been tested extensively in-vivo in several mammalian species (Matsumoto et al (2012) and unpublished observations) and an inflammatory response has not been apparent in small and large animal models with the exception of gout-like inflammation following direct injection into sheep joints. Gout is caused by the presence of crystals in joints, so this result was not unexpected. Lack of an inflammatory response for foreign proteins is not unusual and many foreign proteins are in routine clinical use: For example, silk fibroin protein from the silk worm *Bombyx mori* is commonly used for surgical stitching and Botulinum toxin is widely used in cosmetic procedures.

## Characterization of the active protein

Ultimately, the utility of PODS® depends on the biological activity of the active protein cargo. The active protein may also be analyzed using standard characterization techniques. PODS® crystals first require breaking down, either by proteolytic or alkaline degradation, to release the active protein.

In our experience, most antibodies that detect standard recombinant proteins will also detect the same proteins released from PODS® crystals. However, there are some cases where the tag used to attach the protein to the polyhedrin crystal may modify or obscure the epitope.

### ELISA assays

ELISA assays have been successfully used to monitor the amount of free available active protein released into cell culture media. See for example Nishishita et al (2011).

### Western blots

PODS® crystals may be dissolved by incubation in alkali. Prepare a buffer containing PBS (pH 11) and 1% SDS. Incubate at 65°C for one hour. Add 1 µl of protein loading buffer for each 4 µl of dissolved PODS® crystals.

### Immunoaffinity

PODS® crystals can be readily attached to plastic surfaces. The presence of the active protein can be confirmed by performing an ELISA-like assay directly using a detection antibody against intact PODS® crystals and using Empty PODS® crystals (available from Cell Guidance Systems) as a control.

## Applications of PODS®

### In-vivo delivery

PODS® provide excellent delivery devices for the long-term, localized delivery of growth factors and other proteins with potential medically utility. PODS® growth factors are sold for research use only. However, if you are considering the development of a protein therapeutic, please contact us for support.

### 3D cell culture, organoid culture, bio-inks

PODS® are very robust and can be efficiently incorporated into hydrogels to slowly secrete their cargo protein. PODS are stable in hydrogels for extended periods.

### Localized deposition

PODS® can be readily attached to surfaces and provide a depot for growth factors which are secreted into surrounding media at physiologically relevant concentrations. PODS® may be localized by spotting using a pipette. A 0.5 µl aliquot forms a disc around 2 mm across. Alternatively, PODS® crystals may be combined with a hydrogel. This reduces spreading and slows release. As PODS® crystals are heavier than water, they will accumulate in the base of V well dishes or hanging drops, widely used for embryoid body formation. There are a range of printing techniques which may be applicable to PODS® crystals.

### Generation of gradients

Where PODS® crystals have been localized on a surface, it is possible to generate a biologically effective gradient simply by eliminating any agitation during the period of culture. Such gradients can be maintained for weeks allowing the modelling of complex developmental programs.

# References


- Matsumoto et al. (2012) Bone regeneration by polyhedral microcrystals from silkworm virus **Scientific Reports** 2:935.
- Zakrzewska et al. (2009) Increased Protein Stability of FGF1 Can Compensate for Its Reduced Affinity for Heparin, **The Journal of Biological Chemistry** 11;284(37):25388-403.
- Nishishita et al (2011) The use of leukemia inhibitory factor immobilized on virus-derived polyhedra to support the proliferation of mouse embryonic and induced pluripotent stem cells. **Biomaterials** 32(14):3555-63.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Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*.

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- Purified Exosomes
- Services

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