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# INSTRUCTIONS FOR USE

## OPTIMATE OA

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### 1. PRODUCT DESCRIPTION

The Pregenerate Optimate OA is a sterile general IVD (in vitro diagnostic device) for personalized cartilage analysis.

The Chip (Figure 1) fits the dimensions of a standard 96 well plate and allows the handling of 32 samples simultaneously in terms of loading, medium exchange, and real-time monitoring through microscopy. The volume of each chamber is 8.06  $\mu$ l and the volume of the media channel is 36.92  $\mu$ l. Automation of standard procedures such as loading and medium exchange is possible.

Product Information	Specifications
Material	PS Styron MED 2678
Storage	Store at 10-30°C and 30-65% r.F(humidity), no direct sunlight (UV light)  Once package is open, use immediately.
Shelf-life	The product has a shelf life of 45 days in the original packaging, provided that the single package is not exposed to wet conditions or physical breach of any kind.



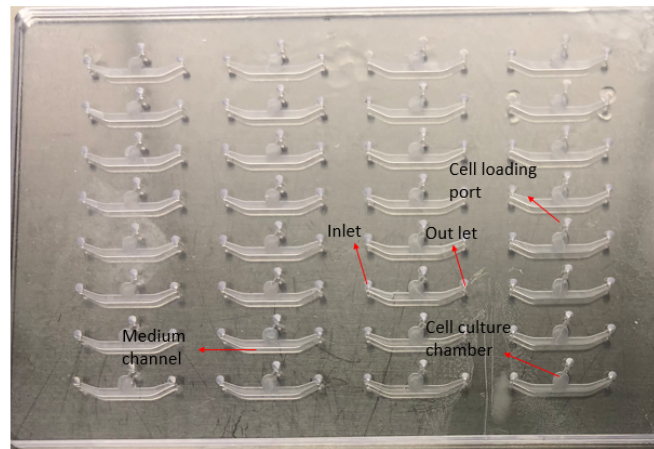
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**Figure 1:** Illustration of Pregenerate's Chip outlining its main features (inlet, outlet, cell loading port, cell culture chamber, medium channel) and design to accommodate 32 different samples simultaneously

## 2. INTENDED USE

Pregenerate's Optimate OA sterile device is suitable to be used for in vitro diagnostic procedures and examinations that require the in vitro culturing of articular cartilage cells in a 3D environment (Rosser et al 2019, Sun et al 2011).

The intended use of the Pregenerate Optimate OA is to allow the in vitro culturing of articular cartilage cells in a 3D environment that mimics avascular cartilage (Rosser et al, 2019), thus being intended for the examination of specimens coming from tissue donations derived from the human body for the purpose of providing information and monitoring therapeutic measures with respect to cell viability.

The Pregenerate Optimate OA allows the in vitro culturing of articular cartilage cells in a 3D environment at a density of 1500 to 24000 cells per  $\mu\text{l}$  with



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the optimal at 3000 cells per  $\mu\text{l}$  for a period of up to 10 days after cell loading. During the culturing time, the cells maintain articular cartilage relevant characteristics including structural organization, cellular morphologies, and gene expression patterns, as well as limited proliferative capacity and low metabolic activity; on-chip-established cartilage microtissues have the ability to react to biochemical injury using inflammatory mediators and also respond to selected therapeutic treatments.

Pregenerate's Optimate OA sterile device allows the in vitro culturing of articular cartilage cells in a 3D environment, akin to avascular cartilage. This allows the monitoring of cell morphology and cell viability via microscopy during the use of the device. It also allows the harvesting of the cells for biochemical assays such as gene expression analysis. Consequently, the culturing of articular cells in the Pregenerate Optimate OA device can serve as a basis for qualitative, semi-quantitative, and quantitative assays (non-exhaustive examples: microscopy, gene-expression analysis, sequencing applications) to provide information about the specimen (human chondrocytes) analyzed.

### **3. PRESENTATION, STORAGE, AND HANDLING**

- The chip is delivered as a carton box containing 30 single-use items.
- Each single-use item is packed individually in a sterilized pouch. To keep sterile, open and use inside a laminar flow tissue culture hood or under similar sterile conditions.
- Store sealed pouches and boxes in a cool dry place between 10-30°C and 30-65% humidity.



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- Exposure to temperature and/or humidity outside the specified conditions may cause inaccurate results.
- Do not freeze or refrigerate.
- Use the chip at temperatures between 15-38°C. According to the datasheet the PS Styron MED 2678 the heat deflection temperature is 82°C tested according to ISO 75-2/A and Vicat softening temperature is 93°C tested according to ISO 306/A 120.
- Use the chip between 30-65% humidity.
- Dispose of chip in accordance with Biosafety Level 2 waste disposal protocols.
- Keep away from thermal ignition sources.
- No direct exposure to the Sunlight.



**The chip should only be used by laboratory personnel trained in Biosafety Level 2 laboratory practices, experienced in standard tissue culture operations, and trained in the use of the standard laboratory equipment listed in section 4 below.**

## 4. SAFETY INFORMATION

- The device must be used by trained technicians with experience in working in Biosafety Level 2 (BSL-2) laboratories
- The device must be used under sterile working conditions
- Once the cells are extracted from the device, the device must be disposed of as a BSL-2 biological material waste
- All consumables used together with the device must be disposed of as a BSL-2 biological material waste

## 5. MATERIALS REQUIRED BUT NOT PROVIDED

- Laminar flow hood (ThermoFisher Scientific)



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- CO2 incubator (Binder)
- Fibrinogen (Sigma, catalog number: F8630)
- Thrombin (Sigma, catalog number: T4648)
- CMFDA (Fisher Scientific, catalog number: C2925)
- Calciumchlorid (Fisher Scientific, Catalog number:11313867)
- Standard cell culture reagents: Hams F12 (Fisher Scientific, Catalog number: 31331028), FBS (Sigma, catalog number: F9665), Anti/Anti (Gibco, catalog number: 15240062), Trypsin (Sigma, Catalog number T4049), PBS (Gibco, catalog number : 10728775)
  - Human Chondrocyte medium: Haems F12 Media (11765-05) supplemented with 1x Antibiotic-Antimycotic (Thermo 15240062) and 10% Fetal Bovine Serum (Sigma Aldrich F9665)
  - CMFDA medium: Haems media supplemented with 5uM CMFDA(Stock of CMFDA with DMSO)
- Microscope ( EVOS M7000, AMF7000, ThermoFisher Scientific)



**Store and use additional material in accordance with the manufacturers' instructions. Inappropriate storage and use of material can affect the performance of the device.**

## 6. PROCEDURE

- Patient cartilage samples may be obtained only by an attending and appropriately licensed surgeon, respectively. Samples must be obtained under sterile conditions and aseptically stored in physiologic solution immediately and throughout transport to PreGenerate GmbH under sealed, cooled (min 4 degrees C) or room temperature conditions. Upon receipt by PreGenerate gmbH, chondrocytes are isolated from the patient cartilage sample obtained



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during surgery (Rosser et al, 2019). The cells are grown in normal cell culture flasks until they are 80 % confluent.



**Patient-derived samples are potentially infectious and must be treated as such according to Biosafety Level 2 protocols.**

- In the next step, the patient cells are harvested and prepared to be loaded to the chip at a loading cell density range of 1500 to 24000 cells per  $\mu\text{l}$  with the optimal at 3000 cells per  $\mu\text{l}$
- CMFDA staining (optional, for microscopic monitoring) Original medium is removed, and cells are incubated for 30min in medium supplemented with 5 $\mu\text{M}$  CMFDA (Thermo Fisher Scientific C2925).
- Cells at the required density are mixed 3:2 with Fibrinogen (SIGMA F8630) and then 1:1 Thrombin (T7513 Sigma, 100U/ml diluted with 40mM Calciumchlorid (Alfa Aesar(35686) 1:50) and then 12 $\mu\text{l}$  are loaded in each Chip.
- After all the chambers are loaded, the cell loading port is sealed using a PCR foil and is kept in CO<sub>2</sub> incubator for 15-30 minutes so that the cell matrix polymerizes.
- In the next step, a medium is introduced through the medium channel using medium inlet until the complete channel is covered. The inlets and outlets are sealed with parafilm. The medium is changed every second day.
- Optionally, a treatment can be added after 2 days.
- Harvest of cells after a maximum of 8-10 days.

## 7. WARNINGS, PRECAUTIONS, AND LIMITATIONS

- Do not use the chip beyond the expiration date (printed on the chip itself, the pouch, and the box).

All expiration dates are printed in Year-Month-Day format.



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- Do not use the chip if the sterile pouch is damaged, broken, unintentionally opened before use in a non-sterile setting, or exposed to environmental conditions outside of those specified.
- Do not use any damaged or broken chips.
- Do not freeze or refrigerate. Use the chip at temperatures between 15-38°C.
- Use the chip between 30-65% humidity.
- For single use only. Do not reuse the device.
- For in vitro diagnostic use only.
- Specific training and guidance is recommended if operators are not trained in working in Biosafety Level 2 (BSL-2) laboratories.
- Wear protective gear such as laboratory coats, disposable gloves, and eye protection while using the chip in conjunction with biological samples.
- Wash hands thoroughly after handling.

## **8. ACCIDENTS REPORTING**

In case of any adverse reactions or events as well as any complaints regarding the product, please contact

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## **9. REFERENCES**

- Rosser et al, Microfluidic nutrient gradient-based three-dimensional chondrocyte culture-on-a-chip as an in vitro equine arthritis model, Mater Today Bio (2019); PMID: 32159153



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- Sun et al, Tissue engineering of cartilage, tendon and bone, Frontiers of Medicine (2011); , PMID: 21681676

**10. INDEX OF SYMBOLS**

	Date of manufacture
	Use by
	Lot number
	Serial number
	In vitro diagnostic medical device
	Sterilized using radiation
	Single sterile barrier system
	Do not use if package is damaged and consult instructions for use
	Keep dry
	Temperature limit








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	Do not reuse
	Consult instructions for use or consult electronic instructions for use
	Caution



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