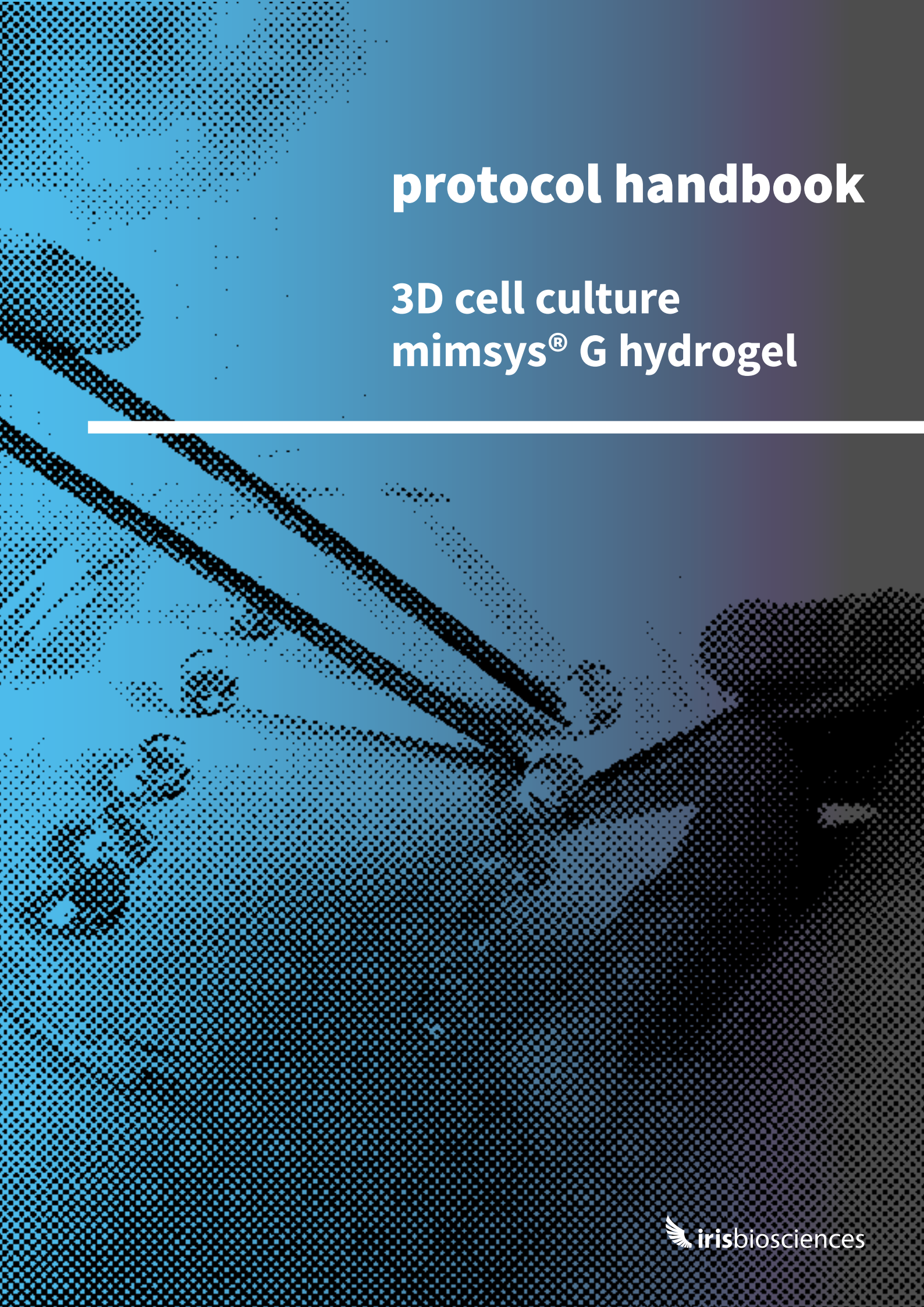
protocol handbook

3D cell culture
mimsys® G hydrogel



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Supporting real discovery

mimsys® G protocol handbook

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01

Cell encapsulation in hydrogels

mimsys® G hydrogels are suitable for 3D cell culture, due to their permeability and biocompatibility characteristics. *mimsys® G* hydrogel provides a highly hydrated microenvironment that resembles tissue extracellular matrix, allowing encapsulated cells to preserve the predisposed biochemistry and phenotype.
Additionally, the transparency of *mimsys® G* hydrogel makes it suitable for established laboratorial cell assays, such as microscopy evaluations, viability protocols, fluorescent stainings and histological procedures.

This protocol is a guideline for cell encapsulation, with tested reproducibility, ensuring homogeneous cell distribution within *mimsys® G* 3D hydrogel.

MATERIALS

· *mimsys® G*

· Sterile distillated water (warm, 37ºC)

· Cell culture media (warm, 37ºC)

· Water-bath (37ºC)

· Positive displacement micropipette or syringe

· 48 well-plate (non-adherent)

· Cells

PROCEDURE

Under aseptic conditions

1. Reconstitute *mimsys® G* with 9 ml of warm, sterile distillated water. Allow it to dissolve in 37ºC water-bath.
2. Prepare a cell suspension of 3 x 106 cell in 300 µl of cell culture media.
3. Add 2.7 ml of *mimsys® G* to the cell suspension and quickly mix by pipetting up and down (hydrogel concentration is 2% w/V, cell density is 1 x 106 cell/ml).
4. Pipette 50 µl of cellular hydrogel to each well.
5. Cover the hydrogel with 500 µl of culture media.
6. Incubate the tridimensional culture.
7. Change cell culture media every 48 hours.

IMPORTANT NOTES

Hydrogel gelation is initiated by the addition of cell culture media. After cell suspension mixture into *mimsys® G* quickly start transferring the cellular hydrogel to the well-plate.

Hydrogel concentration can be optimized based on the cell type mechanical needs.

Cell densities tested within the hydrogel ranged from 1 x 106 to 10 x 106 cell/ml.

Hydrogel volume tested ranged from 50 µl to 150 µl.

The use of positive displacement micropipettes ensures accuracy and precision when pipetting.

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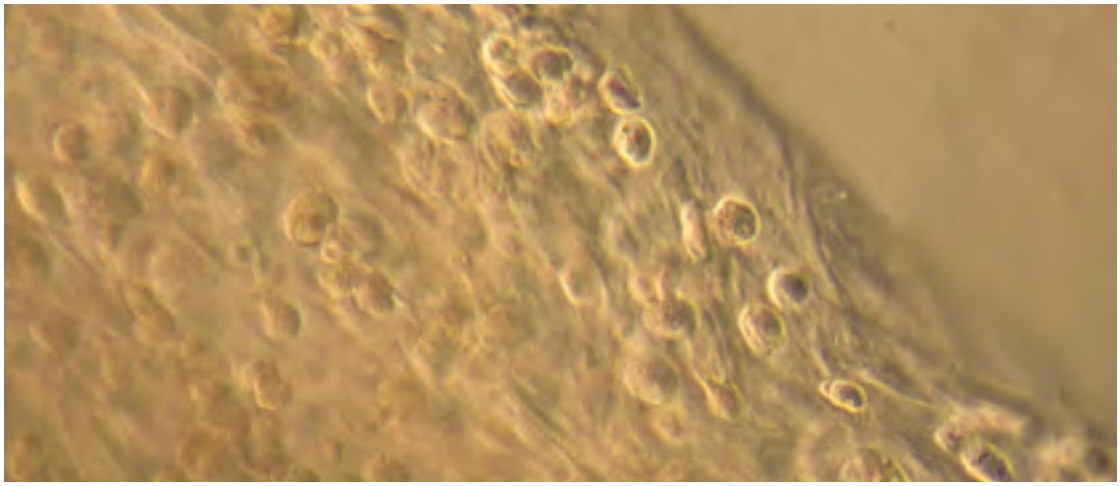
EXAMPLE RESULTS



Example of bright field optic microscope image of L929 cells encapsulated within *mimsys® G* hydrogel prepared as described in protocol

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02

Cell viability by MTS assay

mimsys® G hydrogels with encapsulated cells, are fully compatible with metabolic assays due to its permeability characteristics. MTS is a one-step version of MTT assay. MTS assay is an easy-to-use colorimetric method based on MTS tetrazolium reduction to colored formazan. MTS reduction is accomplished by dehydrogenase enzymes in metabolic active cells. Absorbance of the 3D cell culture supernatant is directly proportional to the number of viable cells.

This protocol is a guideline that has been tested to quantify viable cell activity in *mimsys® G* 3D hydrogel.

IMPORTANT NOTES

Protect MTS solution from the light during procedure execution.

Optimization can be necessary for incubation time, depending on cell type and growth rate.

MATERIALS

· Phosphate Buffer Saline (PBS)

· MTS reagent (*Promega*)

· Cell culture media (warm, 37ºC)

· MTS working solution: 1:5 ratio of MTS in culture media (protect from light)

PROCEDURE

1. After culture, hydrogels with encapsulated cells are washed 2x in PBS.
2. Transfer hydrogels to a new 48 well-plate (one 50 µL hydrogel/well), leaving one well empty.
3. Add 500 µL of prepared MTS reagent to each well of the 48 well-plate, including the empty one (blank).
4. Incubate 2 hours at 37 ºC and 5% CO_2.
5. Mix the well-plate and transfer 100 µL of supernatant to a 96 well-plate, in triplicate.
6. Read absorbance at 490 nm.
7. Subtract absorbance value of blank to the value obtained for each sample.

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03

Live/Dead assay to assess cell viability

mimsys®G hydrogels are transparent, a characteristic compatible with several cell characterization assays. Cell imaging, for instance, is possible by the use of both transmitted light and fluorescence microscopes, using several fluorescent cell stainings. Given the 3D nature of the hydrogel, we recommend the use of confocal microscope for improved imaging.

This protocol is a guideline that has been tested to assess cell viability in *mimsys® G* hydrogel.

IMPORTANT NOTES

Ensure hydrogels are submerged within solutions at all stages. Protect from light.

If you have a large number of samples, stain only 2–3 samples to image at each time to avoid cell death.

Parameters, such as concentrations and incubation times, might require optimization for your specific experimental conditions.

MATERIALS

· Phosphate Buffer Saline (PBS), warm (37°C)

· Calcein AM (1 mg/ml, *Invitrogen*)

· Propidium Iodide (1 mg/ml, *Invitrogen*)

· Staining solution: 1 µL of calcein AM and 3 µl of propidium iodide in PBS (protect from light)

PROCEDURE

1 After culture, hydrogels with encapsulated cells are washed 2x in PBS.

2 Incubate in staining solution for 5 min at 37°C. Wash with PBS to remove unbound reagents.

3 Observe hydrogels with encapsulated cells under a fluorescence microscope as soon as possible: live cells will show green-fluorescent calcein, while dead cells will be detected by red propidium iodide binding to DNA.

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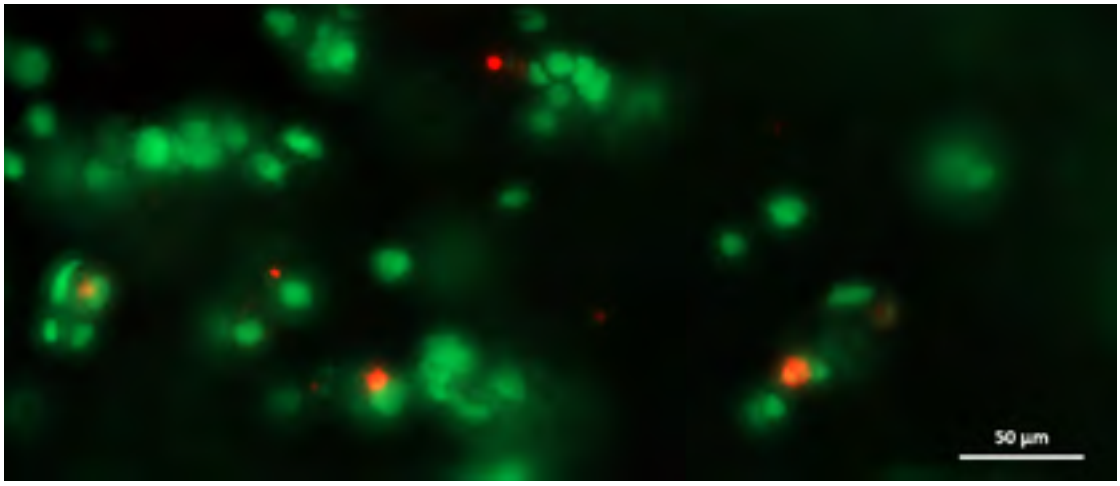
EXAMPLE RESULTS



Example of safranin O staining of cartilaginous matrix (red) produced within 3D cell cultured *mimsys® G* hydrogel.

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04

Fluorescent staining to assess cell morphology

mimsys G® hydrogels are transparent, a characteristic compatible with several cell characterization assays. Cell imaging, for instance, is possible by the use of both transmitted light and fluorescence microscopes, using several fluorescent cell stainings. Given the 3D nature of the hydrogel, we recommend the use of confocal microscope for improved imaging.

This protocol is a guideline that has been tested to assess cell morphology in *mimsys® G* hydrogel.

IMPORTANT NOTES

Parameters, such as concentrations and incubation times, might require optimization for your specific experimental conditions.

Ensure hydrogels are submerged within the different solutions at all stages.

MATERIALS

· Phosphate Buffer Saline (PBS)

· Fixation solution: 10% buffered formalin
(4% solution of formaldehyde in PBS)

· Permeabilization solution: 0.1% TritonTM X-100 in PBS

Ensure manufacturer instructions:
stains are usually prepared and stored at higher concentrations and diluted to working concentration immediately before use.

· F-actin filament staining solution
Example: prepare a working solution of Phalloidin-TRITC (*Sigma*, Ex 510-545/Em 570-573nm) at 50µg/ml in PBS.

· Nucleus staining solution
Example: prepare a working solution of DAPI (*Sigma*) at 0.5µg/ml in PBS.

PROCEDURE

1. After culture, hydrogels with encapsulated cells are washed 2x in PBS.
2. Incubate in fixative solution for 30 min room temperature. Wash 2-3x with PBS.
3. Permeabilize the cells by submerging the gels in permeabilization solution for 5 min RT. Wash 2-3x with PBS.

Protect hydrogels from light beyond this stage

4. Incubate in F-actin staining solution for 1h at RT. Wash extensively with PBS to remove unbound conjugate.
5. Add nucleus staining solution for 5 min at RT. Wash extensively with PBS.
6. Observe hydrogels with encapsulated cells under a fluorescence microscope.

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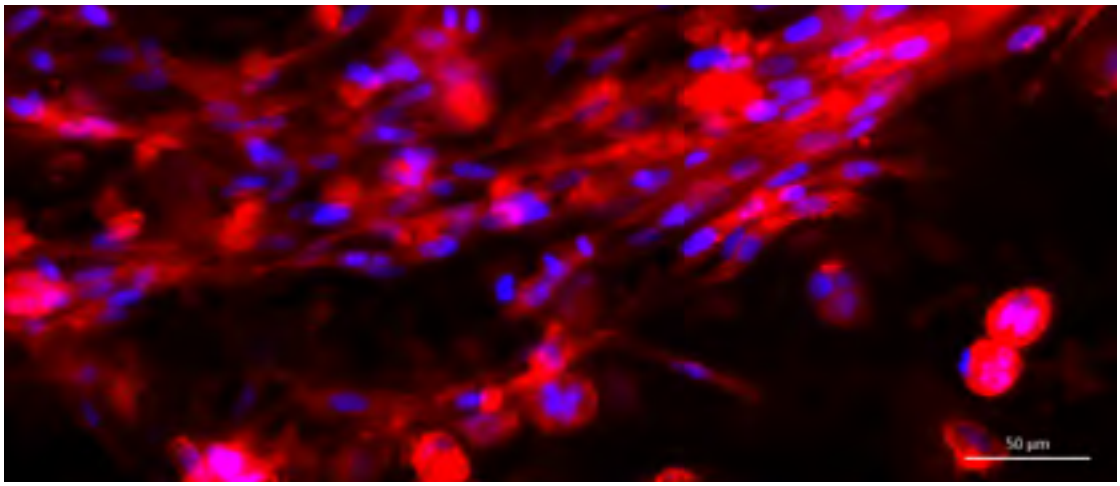
EXAMPLE RESULTS



Example of human *Mesenchymal Stem/Stromal Cells* (Adipose Derived) (Ref. 060231) encapsulated in *mimsys® G* hydrogel, stained with Phalloidin/DAPI.

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Histological analysis

mimsys® G hydrogels are compatible with standard paraffin histology process. A wide range of cell and matrix components within mimsys G® hydrogels have been identified by using common staining protocols.

This protocol is a guideline that has been tested to quantify viable cell activity in *mimsys® G* 3D hydrogel.

MATERIALS

· Phosphate Buffer Saline (PBS)

· Fixation solution: 10% buffered formalin

· Plastic cassettes for standard paraffin processing

PROCEDURE

1 After culture, hydrogels with encapsulated cells are washed 2x in PBS.

2 Incubate in fixative solution for 30 min room temperature.

3 Place samples in plastic cassettes and proceed to standard paraffin processing protocols:

· Processing - dehydration, clearing, and infiltration with paraffin;

· Paraffin embedding;

· Sectioning onto glass microscope slides;

· Staining and mounting according to specific staining standard protocol.

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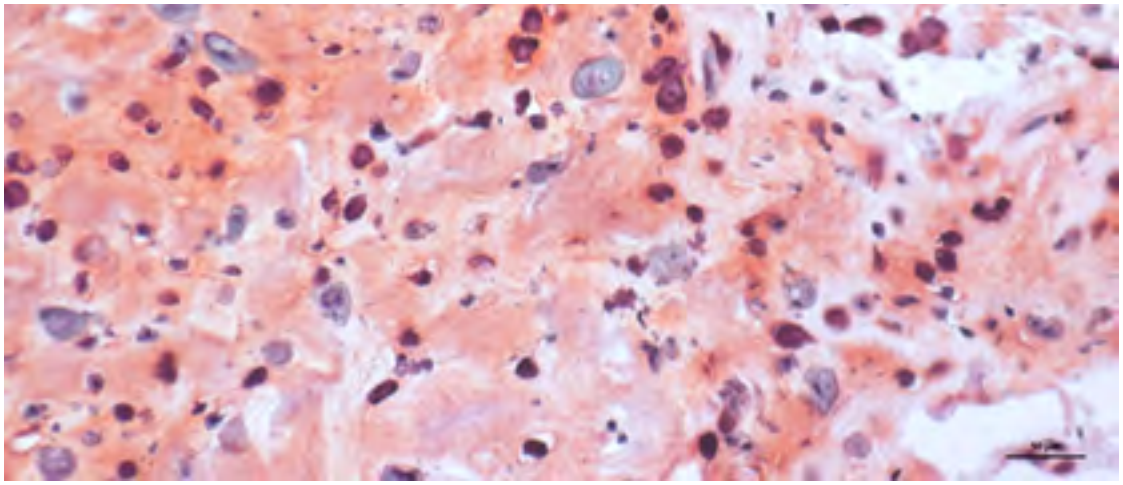
EXAMPLE RESULTS



Example of safranin O staining of cartilaginous matrix (red) produced within 3D cell cultured *mimsys® G* hydrogel.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