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**Protocol status:** Working  
We use this protocol and it's working

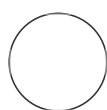
## Human myocardium decellularization V.3

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### ABSTRACT

The protocol represents a step-by-step method to obtain a decellularized cardiac matrix through the combination of sodium dodecyl sulphate (SDS) and Triton X-100. Briefly, cardiac samples obtained from left ventricles of explanted, pathological human hearts were dissected and washed to remove residual body fluids. Samples were then snap-frozen and sliced by a cryostat into 350 µm thick sections. The sections obtained were decellularized using a solution containing 1% Triton X-100 and 1% SDS in combination, for 24 hours, until observing the color change from brownish-red to translucent-white. As a result, the protocol shows efficiency in preserving extracellular matrix architecture and protein composition during the whole process, suggesting that it is worthwhile, highly reproducible and produces a well-preserved decellularized extracellular matrix from cardiac samples.

(The [last step](#) contains a supplemental video with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.)

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PROTOCOL integer ID:  
80556

# Preparation of decellularizing solution


50m

## 1 Preparation of 600 mL of decellularizing solution

50m

1.1 Prepare 300 mL of 2% Triton X-100 solution by measuring  294 mL of double-distilled water in a graduated cylinder and transferring it to a 500 mL beaker.

2m

1.2 Add  6 mL of Triton X-100 to the beaker containing the double-distilled water using a serological pipette.

2m



 Triton X-100 Merck MilliporeSigma (Sigma-Aldrich) Catalog #X100-1L

### Safety information

It is recommended to wear personal protective devices.

## 1.3

15m

### Equipment

Heating Magnetic Stirrer

NAME

VELP SCIENTIFICA

BRAND

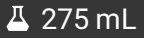
VP-F20520162

SKU

<https://www.velp.com/en-ww/are-aluminum-hot-plate-stirrer.aspx>

LINK

Add a stir bar into the beaker and place it on a magnetic stirrer to mix the solution until completely dissolved.

- 1.4 Prepare 300 mL of 2% SDS solution by measuring  275 mL of double-distilled water in a graduated cylinder and transferring it to a 500 mL beaker.

2m

1.5



5m

#### Equipment

Explorer Pro Precision EP413

NAME

Precision balance

TYPE

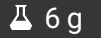
Ohaus

BRAND

80108921

SKU

<https://us.ohaus.com/en-US/Products/Balances-Scales/Precision-Balances/Explorer-Pro-Precision/EP413> <sup>LINK</sup>

Weigh  6 g of SDS powder in a weighing boat using a spoon and an electronic balance. Transfer the powder to the beaker containing the double-distilled water.

 Sodium dodecyl sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #62862**

#### Safety information

This step should be performed under chemical hood wearing personal protective devices.

- 1.6 Add a stir bar into the beaker and place it on a magnetic stirrer to mix the solution until completely dissolved.

10m

- 1.7 Pour the solution in a graduated cylinder and adjust the volume to 300 mL by adding double-distilled water.

4m

1.8



Pour 2% Triton X-100 and 2% SDS solutions, previously prepared, in a 1 L cylinder to obtain a total volume of 600 ml of 1% decellularizing solution. Cover with parafilm and gently mix by inversion to obtain a homogeneous solution.

5m

#### Equipment

##### Parafilm M

NAME

Thermoplastic film

TYPE

Sigma-Aldrich

BRAND


P7793-1EA

SKU

<https://www.sigmaaldrich.com/IT/it/product/sigma/p7793>

LINK

1.9

Transfer 1% decellularizing solution in a 1 L graduated bottle using a funnel to reduce foaming. Store at  +4 °C until use.

5m

#### Note

The final volume of the decellularizing solution can vary according to the number of samples to decellularize. The volume reported in the protocol is intended for 15 samples.

## Preparation of 1x phosphate buffered saline (PBS) solution

29m

### 2 Preparation of 500 mL of 1x PBS

29m

2.1

Weigh all the salt powders in recommended amounts using an electronic balance, a spatula and a spoon.

5m

 0.1 g Potassium Phosphate Monobasic

- 0.1 g Potassium Chloride
- 4.0 g Sodium Chloride
- 0.575 g Sodium Phosphate Dibasic

Transfer the salts into a 500 mL beaker.

- Potassium Phosphate Monobasic Contributed by users Catalog #P5655-1Kg
- Potassium Chloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9333
- Sodium Chloride Merck MilliporeSigma (Sigma-Aldrich) Catalog #S7653
- Sodium Phosphate Dibasic Merck MilliporeSigma (Sigma-Aldrich) Catalog #S9763 1Kg

2.2 Take a graduated cylinder to measure 400 mL of double-distilled water and pour it into the beaker. 2m



2.3 Add a stir bar and place the beaker on a magnetic stirrer to completely dissolve the salts. 15m

2.4 Pour the solution in a graduated cylinder and adjust the volume to 500 mL by adding double-distilled water. 2m

2.5 Check the pH value and adjust to pH 7.4 if needed. 5m  
Store at +4 °C until use.

## Preparation of antibiotic solution 5m


### 3 Preparation of 10 mL antibiotic solution 5m

- 3.1 Accurately weigh  625 µg Amphotericin B using an electronic balance and add it to a  8 mL pen/strep mixture. Mix vigorously until it is completely dissolved.

3m

 Amphotericin B Merck MilliporeSigma (Sigma-Aldrich) Catalog #Y0000005

 Penicillin-Streptomycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #P4333

- 3.2 Pour the solution in a graduated cylinder and adjust the volume to 10 mL adding pen/strep mixture.  
Store at  +4 °C until use.


2m

## Preparation of samples and decellularization procedure

1d 13h 16m

### 4 Preparation and decellularization of samples

1d 13h 16m

- 4.1 Identify and wash the cardiac tissue samples obtained from explanted hearts into a plastic tray using a  0.9 Mass / % volume Sodium Chloride isotonic solution to remove any residual fluid.

10m



 Sodium Chloride Solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #S8776

### 4.2

5m

#### Equipment

##### Dissecting Board

NAME

Board for Anatomical Dissection

TYPE

VWR

BRAND

100498-398

SKU

<https://us.vwr.com/store/product/12359741/dissecting-boards-electron-microscopy-sciences>

LINK

Prepare a set of large surgical scissors, long forceps, fine forceps and scalpel needed to dissect the heart. Use a dissecting board with graduations to measure sample size.

4.3

Cut unrefined samples from full-thickness left ventricle wall avoiding injured areas and wash with **IMJ 0.9 Mass / % volume Sodium Chloride isotonic solution** .

10m



4.4

Place them on the dissecting board and cut, by a dissecting scalpel, 2 cm x 2 cm (length by width) fragments using the graduation on the dissection board as a reference.

15m



#### Note

Fragments should not be larger than 2 cm wide by 2 cm long.

4.5

Snap freeze at **🌡️ -80 °C** .

1h

4.6

1d 13h 16m

#### Equipment

##### new equipment

Cryostat

NAME

Leica

BRAND

CM1950

SKU

<https://www2.leicabiosystems.com/uk/cryostats-APR19/>



LINK

Mount samples on cryostat chuck and slice them one by one to obtain **➔ 350 µm** thick sections.

#### Note

It is recommended to cut at least three 350-µm-thick sections of each sample, using as a reference the same number of sections of native tissue.

- 4.7

Prepare and label with all the information identifying the samples a 50 mL tube for each section. Add  40 mL of decellularizing solution previously prepared , place one section in each tube.

20m

Note


Make sure the tubes are appropriately locked to avoid solution leakage.

- 4.8





1d

Equipment

Platform Rocker STR6	NAME
Orbital Shaker	TYPE
Stuart Scientific	BRAND
L065	SKU
<a href="https://www.akribis.co.uk/stuart-scientific-platform-rocker-str6">https://www.akribis.co.uk/stuart-scientific-platform-rocker-str6</a>	LINK

Place the tubes on an orbital shaker and start the procedure setting moderate speed of agitation for 24 hours, at  Room temperature .

- 4.9

Replace the decellularizing solution in each 50 mL tube with  40 mL of 1x PBS  and  0.2 mL of antibiotic solution .

30m

- 4.10

Stop the agitation and check the color of the sections.






10m



Note




Samples should shift from the native red to translucent white.



- 4.11** Start the agitation on the orbital shaker at a moderate speed overnight, at  Room temperature  **8h**
- 4.12** Stop the agitation. Replace the solution in each 50 mL tube with  40 mL of double-distilled water.  **30m**
- 4.13** Start the agitation on the orbital shaker at a moderate speed for 30 minutes at  Room temperature **30m**
- 4.14** Stop the agitation. Open each tube and gently dry sections to remove the excess of double-distilled water. **30m**

## Sample storage

30m

- 5** Fix decellularized sections for histological analyses. Store at  +4 °C in a  0 Mass / % volume Sodium Chloride isotonic solution for further cell seeding or snap-freeze at  -80 °C for other applications. **30m**

### Note

A cycle of sterilization under UV is highly recommended before cell seeding, and d-ECM must be rehydrated with an appropriate culture medium prior to use.

## Materials List

- 6** **Addictional materials**

EQUIPMENT	BRAND	CATALOG NUMBER	SPECIFICATION
1 L beaker	VWR	511-0318	Clean and autoclave before use
10 mL serological pipette	Falcon	357551	Sterile, polystyrene
50 mL sterile tubes	Falcon	FC-1 352070	Sterile tubes, polypropylene
10 mL graduated cylinder	VWR	612-1518	Clean and autoclave before use
1L graduated cylinder	VWR	612-1524	Clean and autoclave before use
1 L bottle	VWR	215-1596	Clean and autoclave before use
25 mL serological pipette	Falcon	357525	Sterile, polystyrene
500 mL beaker	VWR	511-0317	Clean and autoclave before use
Dissecting scalpel	VWR	233-5526	Sterile and disposable
Fine forceps	VWR	232-1317	Clean and autoclave before use
Funnel	VWR	221-1861	Clean and autoclave before use
Hexagonal weighing boats size M	Sigma-Aldrich	Z708585	Hexagonal, polystyrene, 51 mm
Hexagonal weighing boats size S	Sigma-Aldrich	Z708577	Hexagonal, polystyrene, 25 mm
Large surgical scissors	VWR	233-1211	Clean and autoclave before use
Long forceps	VWR	232-0096	Clean and autoclave before use
Pipette gun	Eppendorf	613-2795	Eppendorf Easytip® 3
Plastic tray	VWR	BELAH162620000	Corrosion-proof polypropylene
Spatula	VWR	RSGA038.210	Clean and autoclave before use

	EQUIPMENT	BRAND	CATALOG NUMBER	SPECIFICATION
	Spoon	VWR	231-1314	Clean and autoclave before use
	Stir bar	VWR	442-0362	Clean and autoclave before use

## Spotlight video

- 7 (The following video contains extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.)

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