VERSION 3

APR 24, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.4r3l2o22xv1y/v3

External link:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0276224

Protocol Citation: Immacolata Belviso, Anna Maria Sacco, Domenico Cozzolino, Daria Nurzynska, Franca Di Meglio, clotilde.castaldo, Veronica Romano 2023. Human myocardium decellularization. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l2o22xv1y/v3Version created by clotilde.castaldo

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

Human myocardium decellularization V.3

Immacolata Belviso[1], Anna Maria Sacco[1], Domenico Cozzolino[1], Daria Nurzynska[2], Franca Di Meglio[1], clotilde.castaldo[1], Veronica Romano[1]

[1]Department of Public Health, University of Naples Federico II, Naples, Italy;
[2]Department of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, University of Salerno, Baronissi, Italy

University of Naples Federico II - Human Anatomy

clotilde.castaldo

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.)

Created: Apr 14, 2023

Last Modified: Apr 24, 2023

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	LINK

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 shoul 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 untile 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. 2m
Store at +4 °C until use.

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 [M] 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 [M] 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 (lenght 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	TYPE
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
>
> https://www.akribis.co.uk/stuart-scientific-platform-rocker-str6 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. 30m
Store at +4 °C in a [M] 0 Mass / % volume Sodium Chloride isotonic solution for further cell seeding or snap-freeze at -80 °C for other applications.

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 Easypet® 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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