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Human myocardium decellularization V.2

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We use this protocol and it's working

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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.

Protocol materials

- Amphotericin B **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**Y0000005
- Penicillin-Streptomycin **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P4333
- Potassium Phosphate Monobasic **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
- Sodium Chloride Solution **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S8776
- Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**X100-1L
- Sodium dodecyl sulfate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**62862

Troubleshooting

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. 5m

Store at +4 °C until use.

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 **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	NAME
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 go to step #1 , 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 go to step #2 and 0.2 mL of antibiotic solution go to step #3. 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	VWR	612-1518	Clean and autoclave

	EQUIPMENT	BRAND	CATALOG NUMBER	SPECIFICATION
	cylinder			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