

Apr 20, 2024

Version 2

## Procedure for Seeding Cells on the Disque Platform V.2

DOI

[dx.doi.org/10.17504/protocols.io.4r3l24ppjg1y/v2](https://dx.doi.org/10.17504/protocols.io.4r3l24ppjg1y/v2)



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Human Islet Research N...



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**Protocol Citation:** Peter Anthony Jones\*, Kisuk Yang\*, Jeffrey M Karp 2024. Procedure for Seeding Cells on the Disque Platform. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.4r3l24ppjg1y/v2> Version created by **Sandy Beshir**

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** April 20, 2024

**Last Modified:** April 20, 2024

**Protocol Integer ID:** 98528

**Keywords:** zinc content of native islet, screening of zinc, stem cell, procedure for seeding cell, islet replacement therapy, seeding cell, zinc content, lowered zinc content, zinc ion, 3d discs within 2d, zinc, cell niche, increasing cell proliferation rate, high concentration of zinc ion, reliable screening platform, cell, cell proliferation, cell niche in order, 3d disc, inducing prodrug, cell proliferation rate in type, native islet, cell loss, 3d culture system, islet, dyrk1a inhibitor harmine, 2d culture system, activated prodrug

**Funders Acknowledgements:**

NIH

Grant ID: UC4 DK104165

## Abstract

Advances in treating  $\beta$  cell loss include islet replacement therapies or increasing cell proliferation rate in type 1 and type 2 diabetes. We previously developed a proliferation-inducing prodrug (ZnPD6) that targets the high concentration of zinc ions in  $\beta$  cells, and which exhibits a 2.4-fold increase in  $\beta$  cell proliferation compared to the DYRK1A inhibitor harmine. These prodrugs were identified through screening on the Disque Platform (DP)—a high-fidelity culture system where stem cell-derived  $\beta$  cells are reaggregated into thin, 3D discs within 2D 96-well plates that mimic in vivo conditions.

The Disque Platform allows for the formation of 3D micro-tissues within an automation-friendly design, and is capable of systematically manipulating the cell niche in order to identify chemical and physical cues that enhance  $\beta$  cell proliferation. The Disque Platform better replicates the zinc content of native islets, enabling for the screening of zinc-activated prodrugs whose activity cannot be detected in 2D culture systems, which typically display a markedly lowered zinc content. The Disque Platform is a reliable screening platform that bridges the advantages of 2D and 3D culture systems and responds to interventions when conventional systems cannot produce a clear signal or readout. Here we describe a standard protocol for the formation of 3D micro-tissues in the Disque Platform.

# Materials

## Equipment

Laser machining tool with 75-Watt 10-μm CO2 laser	NAME
PLS6MW	TYPE
Universal Laser Systems	BRAND
PLS6MW	SKU
<a href="https://www.ulsinc.com/products/platforms/pls6mw">https://www.ulsinc.com/products/platforms/pls6mw</a>	LINK



## Equipment

1.5 mm thick cast PMMA sheet for Disque and support pedestal	NAME
1.5 mm thick	TYPE
McMaster-Carr	BRAND
8560k171	SKU
<a href="https://www.mcmaster.com/catalog/127/3896">https://www.mcmaster.com/catalog/127/3896</a>	LINK

Equipment

PETE polyester track etched membrane filter	NAME
1.0-µm pore size	TYPE
Sterlitech	BRAND
PET1047100	SKU
<a href="https://www.sterlitech.com/polyester-membrane-filter-pet1047100.html">https://www.sterlitech.com/polyester-membrane-filter-pet1047100.html</a>	LINK


Equipment

Acrylic solvent cement to attach membrane filter	NAME
IPS CORP. SCIGRIP	BRAND
10300	SKU
<a href="https://www.ellsworth.com/products/by-manufacturer/ips-corp/adhesives/solvent-based/ips-corp.-scigrip-3-acrylic-plastic-cement-solvent-based-adhesive-clear-1-pt-can/">https://www.ellsworth.com/products/by-manufacturer/ips-corp/adhesives/solvent-based/ips-corp.-scigrip-3-acrylic-plastic-cement-solvent-based-adhesive-clear-1-pt-can/</a>	LINK

Equipment			
96-well plate			NAME
Corning			BRAND
N/A			SKU
<a href="https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/Assay-Microplates/96-Well-Microplates/Corning%C2%AE-96-well-Polypropylene-Microplates/p/corning96WellPolypropyleneMicroplates">https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/Assay-Microplates/96-Well-Microplates/Corning%C2%AE-96-well-Polypropylene-Microplates/p/corning96WellPolypropyleneMicroplates</a>			LINK

Equipment			
Transwell inserts			NAME
Corning			BRAND
N/A			SKU
<a href="https://www.fishersci.com/shop/products/costar-transwell-permeable-supports-9/p-176666">https://www.fishersci.com/shop/products/costar-transwell-permeable-supports-9/p-176666</a>			LINK


 TrypLE Express **Thermo Fisher Scientific Catalog #N/A**


 Ultrapure Laminin Mouse 1 mg **Corning Catalog #354239**

 Collagen IV Mouse 1 mg **Corning Catalog #354233**


 70% ethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #470198**


 PBS


 SC  $\beta$  cell clusters **Melton Lab**

 Culture media for SC  $\beta$  cells **Melton Lab**

 Human primary islets **Prodo Laboratories, Inc**



 Culture media for human islets: PIM(S) supplemented with PIM(ABS G and 3X) **Prodo Laboratories, Inc**

 incubator 37°C, 5% CO<sub>2</sub>, 100% humidity

 UV sterilizer (UV lamp in biosafety cabinet)




## Troubleshooting

## 1 **DP fabrication**


1. Disques (  3.0 mm inner diameter) were engraved by laser cutter from  1.5 mm thick acrylic sheets.
2. A 1.0µm pore sized-hydrophilic PTFE membrane was attached to the bottom of a Discue using acrylic glue.
3. The reverse side of the membrane was attached to a supporting pedestal engraved by a laser cutter.  
Laser cutter settings: Vector cut - 90% power / 5% speed / 500 ppi
4. The Disques were sterilized by incubation with 70% ethanol overnight and ultraviolet (UV) radiation for 1 hour before placement into the bottom of 96well plates.

## 2 **Preparation of culture plate** (at least two days in advance to seeding)

### ***Coating the DP***

1. Thaw collagen IV and laminin on ice at  4 °C overnight.
2. Break up collagen IV fibers with 23-gauge needles (lubricate needle and syringe with media first).
3. Spin down collagen IV solution at 100g for 5 minutes to pellet remaining large fibres.
4. Collect supernatant – this is what we will use.
5. Make coating solution with concentration of 100 µg/mL of collagen IV and laminin in media.
6. Add  50 µL coating solution to each insert (for 24 well plates).
7. Tap well plate to make sure coating solution covers the bottom of each insert.
8. Incubate inserts in coating solution in a  37 °C incubator for 2-2.5 hours.
9. Wash inserts 2-3 times with PBS.




10. Store the coated Transwell inserts in  4 °C .



### ***Placing the washers into Transwell inserts***

1. Using a tweezer, place sterilized washers into coated Transwell inserts.
2. Make sure the washer is lying flat at the bottom of the insert.

## **3 Preparation of Media and Growth factors**

1. Estimate the amount of media and factors needed.
2. In TC hood, transfer the required amount of media into a Falcon tube. Warm in  37 °C water bath for 10-15 minutes.
3. Meanwhile, thaw the growth factors on ice.
4. Add factors to new media with the correct dilutions.
5. Mix factors in media by pipetting up-and-down.


## **4 Dispersal**

1. Warm up sterile TrypLE solution in a  37 °C water bath.  
**a.\*Tip:** It is recommended that  1 mL TrypLE is used to disperse ~5 M cells.
2. Disperse cell clusters in TrypLE for 10 minutes, pipette to break up the clusters every 5 minutes.
3. During the 5 minute-breaks, prepare media with Rock-I and counter balance for cell suspension.
4. Make sure the cell suspension looks like a homogenous, milky solution.
5. Centrifuge at 250g for 4 minutes to pellet single cells, then remove TrypLE.
6. Add media with Rock-i (1:1000 dilution) to wash cells.
7. Centrifuge at 250g for 4 min. to pellet single cells, remove wash media.








8. Re-suspend cells in media with Rock-i.

**a. \*Tip:** To seed 0.8M cells/well, since each washer holds ~  20  $\mu\text{L}$  of volume, it is convenient to re-suspend dispersed cells at ~40M cells/mL.

## 5 Cell Count

9. Dilute a small sample of cell suspension for cell counting.

**a. \*Tip:** It is convenient to dilute the cell suspension at 1:100 for counting (it makes the math easy). For dilution of Trypan Blue, we use 1:10. Typically, we dilute cells for counting by mixing  1  $\mu\text{L}$  of cell suspension with  89  $\mu\text{L}$  of PBS and  10  $\mu\text{L}$  of Trypan Blue.

10. Count cells on a hemocytometer.

11. Determine the amount of cell suspension to put into each well.

## 6 Seeding

12. Place the appropriate amount of cell suspension into the centre of the washer.

13. Centrifuge at 200g for 4 min to form cell discs.

**a.Note:** used to do it at 250g, but media leaks through the 3um membrane at 250g. Pilot experiments done before to show that centrifugation speed and duration do not affect cell disc thickness, so changed speed to 200g . Now media doesn't leak.

14. Gently add the media with the full recipe of growth factors into and under the insert.

\*If media is not ready upon forming cell discs in well-plate, make sure to cover cell discs with a small amount of media **with Rock-i** (~100  $\mu\text{L}$ ) while they are waiting inside the incubator, so they don't dry out.