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# mPSM protocol V.2

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protocol .

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Protocol to perform 2D ex vivo segmentation assay (Lauschke et al. 2013).

Takehito Tomita 2022. mPSM protocol. **protocols.io**

<https://protocols.io/view/mpsm-protocol-b35dqq26>

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1 Coat dishes/plates with fibronectin.

1h

- 1.1 Dilute fibronectin (Sigma, Cat.No: F1141) with PBS to 50ug/mL (20 fold dilution).
- 1.2 Fill each well of 8-well plate (NuncTMLab-TekTMII Chambered Coverglass, Cat.No: 155409PK) with ~160μl of fibronectin solution and incubate for 1 hour in 37°C.
- 1.3 Remove all fibronectin solution from the well(s) and dry for 1 hour at room temperature (~25°C).

2 Prepare dissection medium and culture medium.

10m

2.1 Mix the following ingredients in a 50 mL falcon tube:

▢ 0.4 g BSA

▢ 400  $\mu$ L glutamine

▢ 35.52  $\mu$ L 45% glucose

▢ 40 mL DMEM-F12

Vortex well.

2.2 Separate ▢ 10 mL from the mix in step 2.1 and filter through a 0.22 $\mu$ m filter (Millex-GV, Cat.No: SLGV033RS) into a fresh 15 mL falcon tube. Add

▢ 100  $\mu$ L Penicillin/Streptomycin (Gibco, Cat.No: 15140-122) to achieve

100U/mL. This mix is used as culture medium. Equilibrate in 37°C 5% CO<sub>2</sub> until use.

2.3 Add ▢ 510  $\mu$ L HEPES to the remaining ▢ 30 mL mix in step 2.1. This mix is used as dissection medium. It can be kept at the bench until use.

3 Wash the dried wells with ~200 $\mu$ L culture medium per well. Apply fresh culture medium (160 $\mu$ L ~ 300 $\mu$ L per well) and equilibrate in 37°C 5% CO<sub>2</sub> until use.

4

20m

Extract mouse embryos and collect tails in dissection medium.

5

Cut the tail bud using a scalpel in dissection medium. Be careful not to flick the tail buds as this damages the tissue.

5m

6

Wash the tail buds once in a dish containing culture medium. Quickly move the tail buds to the fibronectin coated wells. Align the tissue orientation using forceps to place the cut side downward.

1m

7

Incubate in 37°C 5% CO<sub>2</sub> for 1hr to let the tissue attach.

1h

- 8 Place the well into the metal box to fit into the microscope. Add a strip of wet paper towel inside the metal box to prevent drying. Carefully move the box into the microscope, as tissue can detach quite easily.
- 9 Set up imaging.