Stampwell

PROTOCOLS & SPECIFICATIONS

idylle

STAMPWELL V & Z PROTOCOL

Stampwell V: V-shape 300 µm & 500 µm
Stampwell Z: Embryo 1 & 2 and Larvae 1 & 2

The material you need

- ✔ Hydrogel (agarose, alginate, phytagel, methylcellulose ...)
- ✔ 35 mm petri dish, with or without glass bottom
- ✔ A Pipetman or a Pasteur pipette to load samples in wells
- ✔ A cutter blade
- ✔ A non-wounding spatula
- ✔ A stereomicroscope ideally

1. Cleaning

- The first time you use Stampwell, soak it in water overnight under stirring.
- Next time, if you need to work in sterile conditions, clean your stamp with Surfanios™ disinfectant detergent.
- Rinse with water.
- Moreover if bits of dirt are stuck in the grooves, you can use a sonicator.

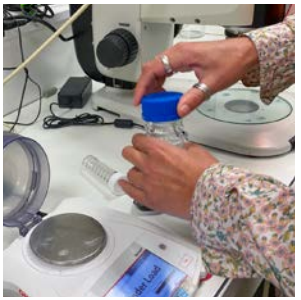
2. Agarose gel preparation

It is suggested to prepare a 2% normal gelling temperature agarose. You can use any other hydrogel of your choice, it only has to be stiff enough once reticulated.

- Ideally, prepare small amounts. 50 mL is more than enough.
- Use a bottle twice as big as the desired volume.
- In a 100 mL glass bottle, weight 1 gram of agarose.

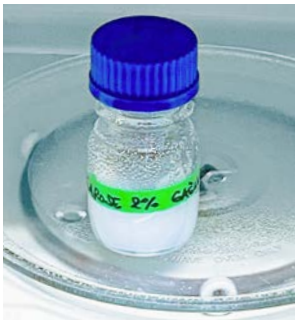
- Add 50 mL of distilled water.

- Close the bottle.



- Autoclave.

- Warm the agarose preparation in a microwave, full power.



- Slightly loosen the cap.
- Place the bottle on the periphery of the microwave plate (not on the center, this is where warming is the less efficient).
- Open the door and gently rotate the solution to spread the liquid agarose on top of the one that is still jellified.
- Repeat every 10 seconds without opening the cap until the agarose is fully dissolved.
- Stop it right away as soon as it boils (before it spills over!).

- Use your agarose gel right away when it is still liquid.

3. Wells imprinting

Pour liquid agarose directly onto the pins of Stampwell.

This will prevent the formation of bubbles.

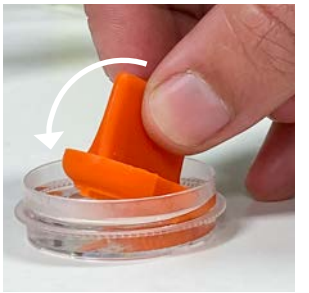
- Pour liquid agarose in the dish.

The level of agarose should reach half of the height of the lateral wall of the stamp, but never flow over it.

- Immediately place the stamp in the dish.

Avoid placing the stamp with a downward pressure.

Prefer a tilting movement (from the edge to the vertical position) until the whole surface of the stamp is in contact with the dish, to avoid the formation of air bubbles.



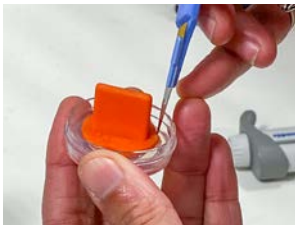
- If you are using a 6-well plate, you can align the stamps in each well to automatize more easily image acquisition with the microscope software.
- Reticulate the hydrogel.

- For an agarose gel, allow the whole set up to cool down: to speed up the process, you can leave it at 4 °C for 5 minutes.
- A neat result is obtained by passing a scalpel blade all around

Stampwell | Protocol

Stampwell | Protocol

the stamp to make sure it is well detached from the gel pad.



Do not remove the stamp vertically.

Option 1: take out the stamp by tilting it a bit at first, then pull firmly.

Option 2: hold the gel pad with a non-wounding spatula to avoid the gel detaching itself from the dish. Then pull the stamp firmly.

4. Samples loading

- Wash the gel.

- For live samples: If you have prepared your gel from water, it is recommended to wash the whole gel with culture medium thrice.
- Keep it at the desired temperature for a few hours, in the incubator for example.
- For fixed samples, operate the same pre-washing step but use the mounting medium.
- Prepare the material to place samples: either a 100-200 µL yellow tip or an embryologist mouth pipette using the following protocol.

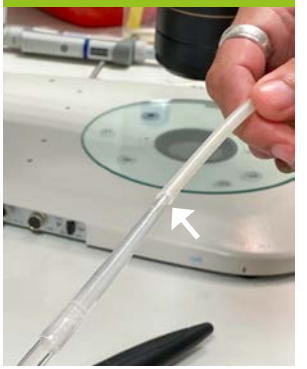
HOW TO MAKE AN EMBRYOLOGIST MOUTH PIPETTE

A. Bring the center of a glass capillary tube into a medium height flame.

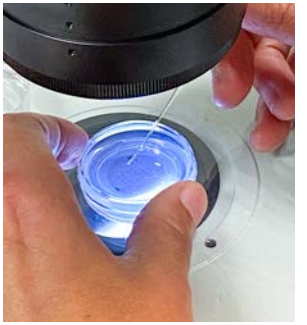
B. As the glass capillary tube begins to melt, quickly lift the tube out of the flame and then pull the tube outward about a few centimeters.

C. The pulled tube is then snapped in the middle, producing two glass transfer pipettes.

D. The glass transfer pipette is then connected to a Tygon® tube with a blue tip at one end a yellow one at the other end to hold it (then, indeed it is on you to decide!).



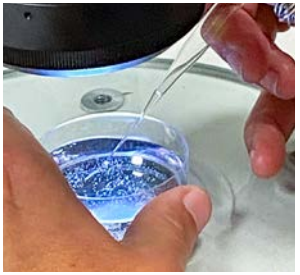
To control where and how each sample is placed in each well, it is highly recommended to use a stereomicroscope.



- Train yourself before with random samples to be familiar with this step.
- Before pipetting your sample, pre-rinse your tip or pipette with

serum or medium. It prevents the samples to stick to the tip (or the glass pipette).

- **Make sure you have pipetted enough medium before taking the samples, so that it will not stick to the air-liquid interface.**
- Take one sample at the time when you start. Once you feel comfortable enough, you can load up to tens of samples. It depends on the length of the straight part of the pipette. Do not forget to space the samples enough in the pipette to avoid mixing them.



- **If you use a Sampwell V-Shape: the conical tip of Stampwell imprints a shape of well which includes a slope. It allows to slide the tip on it. Left-handed people: beware, you might want to rotate the dish so that the slope is on your left side.**
- Do not touch the gel with the tip.
- Once the tip is at the bottom of the well, release the pressure very gently.
- If the flow is too strong, the sample will pop out of the well and will float around in the dish. The sample should almost fall in the well by gravity rather than being pipetted out.

5. Imaging

- If possible, automatize the acquisition: the spacing between wells is standard (See Stampwell specifications). If your samples are homogenous in size, they should be all at the same distance from the bottom of the dish.
- Upon labelling the positions while looking through the eyepieces, you can easily read the number of the well. Hence, you can keep track of the specimen and proceed to further analysis (fixing and staining, or snap freeze and sequence...).
- You can reiterate imaging for several weeks. You may re-use the saved position parameters on the microscope. See "after acquisition" to take care of your samples during these weeks.

6. Manipulating samples

- Operate with a stereomicroscope.
- Gently place the tip to the bottom of the well (with already a small amount of liquid) and suck the sample out.
- For the fish/tissue explants wells, make sure you use smooth forceps and tools not to scratch the gel pad.

7. After acquisition

- Clean Stampwell as previously described. See Cleaning, section 1.
- Either single shot or time lapses, you can take your samples back to the incubator. They will keep on growing in the wells. They will not be constrained.

STAMPWELL U PROTOCOL

Stampwell U: U-shape

1. Material you need

- ✔ Stampwell U-shape
- ✔ Agarose powder
- ✔ Distilled water
- ✔ 35 mm petri dish, or any larger vessel (i.e. 6-well plate)
- ✔ Microwave (if using agarose)
- ✔ Cell suspension & cell culture medium
- ✔ (Optional) sterile spatula to facilitate stamp removal

2. Before use

- **The 1st time you use Stampwell: soak it in water overnight under stirring.**
- Before use, clean the Stampwell with antibacterial or hydroalcoholic solutions. Rinse thoroughly with water.

- **Do not use any organic solvents neither autoclaving as these may alter the stamp material.**

3. Agarose gel preparation

- **It is suggested to prepare a 2% normal gelling temperature agarose. You can use any other hydrogel as long as it provides non-adherent properties and is stiff enough to allow proper microwell formation once reticulated.**
- Weight the desired amount of agarose in a bottle containing twice the final volume prepared.
- Add the required volume of distilled water (i.e. 50mL for 1 g of agarose to prepare a 2% agarose solution).
- Close the bottle and autoclave.
- Warm extemporaneously the agarose preparation in a microwave, full power.
- Unscrew the cap but keep it on.
- Place the bottle on the periphery of the microwave plate (not on the center, this is where warming is least efficient).
- Open the door and gently rotate the solution to spread the liquid agarose on top of the one that is still jellified.
- Repeat every 10 seconds without opening the cap but keeping it on the bottle to allow the pressure due to the steam production to be released, until the whole agarose is dissolved.

- Stop when the first bubbles due to the boiling appear.
- Use it right away when it is still liquid.

4. Wells imprinting

- To prevent the formation of bubbles, pour 1 mL of liquid agarose directly onto the stamp.

- Gently tilt the stamp above the dish to allow the excess liquid to flow.
- Immediately add 1 mL of liquid agarose in the dish.

- Immediately place the stamp in the dish.

Avoid placing the stamp with a downward pressure.

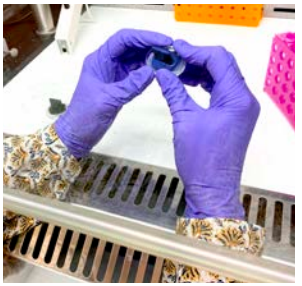
Prefer a tilting movement from the edge to the vertical position until the whole surface of the stamp is in contact with the dish, to avoid the formation of air bubbles.

- Complete the volume of hydrogel to reach half of the height of the stamp's lateral wall.

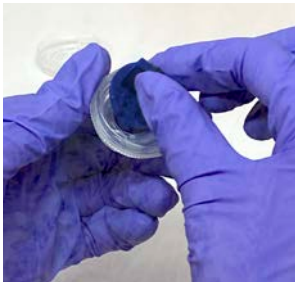
The level of agarose should never spill over.

- Reticulate the hydrogel.

You can apply a gentle pressure on the sides of the dish to start detaching the stamp of the agarose gel.



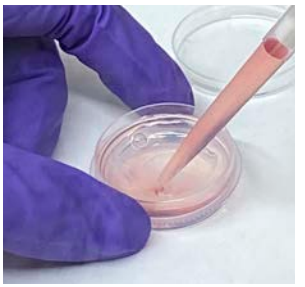
Take out the stamp by tilting it a bit at first and by pulling firmly.



If possible, hold the gel pad with a non-wounding spatula to avoid detachment from the dish.

Do not remove the stamp vertically.

- Rinse the gel with appropriate culture medium:
  - Add 2-3 mL of culture medium.
  - Leave the dish 1h in the incubator to allow for diffusion.
  - Remove the culture medium.



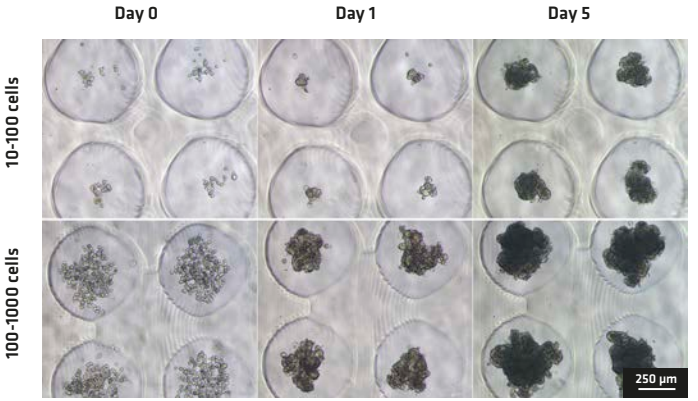
- Repeat the rinsing steps 2 more times or let the culture medium diffuse in the agarose gel overnight.
- Remove the rinsing medium and replace with 1 mL of fresh culture medium.

It is important to let the culture medium diffuse in the agarose gel to improve the survival of the aggregates.

5. Cell seeding

- Prepare the cell suspension following your usual passaging procedure.

DIFFERENT SIZES OF CELL AGGREGATES



Stampwell | Protocol

- Different sizes of cell aggregates or growth dynamics can be induced.
- Adapt the cell suspension concentration according to the number of cells you would like to seed in each microwell (Ncells/microwell).
- There are 81 microwells per stamp.
- Account for the cells that will be seeded in the groove delimitating the Stampwell (around 20%).

These peripheral cells will help conditioning the medium to promote cell survival in low cell number conditions.

- Dilute your cell suspension to obtain : $\frac{N \text{ cells}/\mu\text{well} \times 81}{80\%}$ cells per mL
- If Matrigel® needs to be added with the cells in the microwells, please follow the "Cell seeding in Matrigel®" additional instructions.

Cell seeding in Matrigel®

Beforehand, we recommend cooling the pipette tips that will be used for the manipulation of the Matrigel® and the cell suspension containing the Matrigel® (e.g., storage at -20°C).

At the end of the "Well imprinting" part, cool the dish containing the gel in 1 mL of fresh culture medium at 4°C (e.g., 30 min in a refrigerator).

Prepare the cell suspension following your usual passaging procedure.

Prepare a cold chamber by filling a box with crushed ice to perform the rest of the experiment on ice.

Collect a volume of cell suspension corresponding to the needed number of cells and place it in an Eppendorf tube on ice. Calculation of the needed number of cells are detailed in the standard cell seeding procedure.

On ice, add cold culture medium in the Eppendorf tube to reach a final volume of 800 µL to 950 µL of cell suspension.

On ice and with a cold pipette tip, complete with 200 µL to 50 µL of Matrigel® to obtain 1 mL of final cell suspension. We recommend using 5-20% (v/v) of Matrigel® final concentration depending on your application. Indeed, higher concentrations of Matrigel® limit the cell sedimentation before Matrigel® gelation in the microwells.

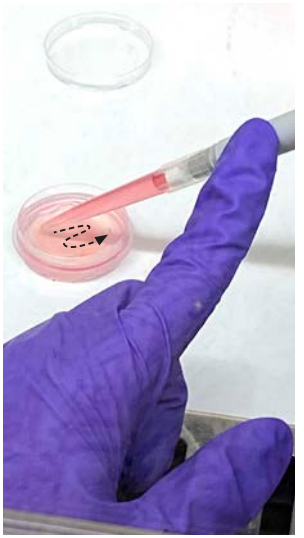
On ice, gently pipette up and down with a cold tip to homogenize the Matrigel®-containing cell suspension.

On ice, gently pipette 1 mL of the cold cell suspension in the cooled microwells as described in the standard cell seeding procedure.

Keep the dish at 4°C to allow for cell sedimentation at the bottom of the microwells before the Matrigel® gelation.

After the cell sedimentation in microwells, place the dish in the appropriate culture environment for your cells (e.g., incubator at 37°C and 5% CO2) allowing the Matrigel® gelation.

- Gently pipette 1 mL of the prepared cell suspension by slowly moving the tip in a serpentine trajectory in the medium above the microwells.



Do not either flush or centrifuge.

- Place the dish in the appropriate culture environment for your cells (e.g., incubator at 37°C and 5% CO2).

We recommend placing the dish in a humidity chamber (e.g., bigger dish with sterile water) to avoid medium evaporation over time.

- Gently add 0.5-1 mL of medium every 3-4 days.
- Use standard bright-field microscopy to image the microwells and track cellular aggregate maturation over time.

6. Sample analysis

- Cells can be imaged directly *in situ*, preferably using an upright microscope equipped with a dipping lens objective. Optical aberrations may be experienced when using an inverted microscope due to the round shape of the wells.
- Samples may be fixed *in situ* and embedded in agarose for downstream analyses (i.e. immunostaining or histological cuts).

Culture medium components will diffuse within the agarose gel. When adding external factors to the cultured cells (i.e. drugs, antibodies, etc), remember to adjust their concentration by taking into account the agarose gel volume.

Alternatively, 3D cell structures can be collected from the wells for downstream analyses:

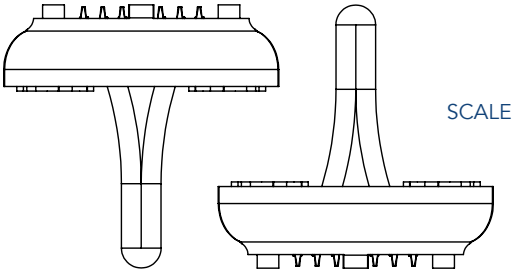
- Remove the cells aggregated in the grooves at the periphery of the gel.

They formed a solid ring, easy to remove.

- Flush cells using a micropipette.

You may either flush all spheroids at once for bulk analysis using a P1000 pipette tip and flushing at the dish periphery, or dislodge individual spheroids using a P200 pipette tip and flushing near the well. Performing this step under a microscope will help ensuring proper spheroid retrieval.

STAMPWELL SPECIFICATIONS



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All shapes

Manufacturing process: we produce countertyped silicone molds to manufacture polyurethane stamps.

External diameter: 26 mm. It is compatible with almost all 35-mm dishes.

Distance between the wells bottom and the dish bottom (except for U-Shape): 100 µm. Three feet have been designed so that Stampwell can lay on the glass of almost all glass bottom dishes, whatever their size. It ensures that the bottom of each well is at the very same distance from the bottom of the dish.

Demolding: is made easy thanks to an ergonomic handle and curvature of the stamp's base, as well as a notch allowing an object with a rounded tip to pass through to hold the hydrogel during demolding.

Lifetime: each stamp can be used dozens of times.

STAMPWELL **V**

V-shape 300 µm - for the imaging and culture of medium-size rounded 3D objects such as spheroids and organoids

Number of pins: 42

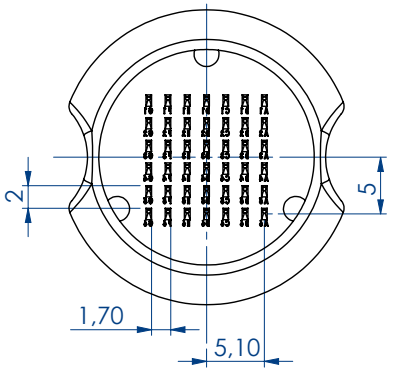
Pins imprint wells with the following dimensions:

- **Depth of wells:** 1 mm
- **Wells bottom diameter:** 300 µm - **Shape:** circular
- **Wells upper side:** 1 mm * 0,5 mm - **Shape:** rectangular
- **Spacing between 2 wells:** 1,70 mm on the lines and 2 mm on the columns

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

The right edge of the wells provides a slope to support and guide the pipette cone when loading the samples.

V-shape 300 µm



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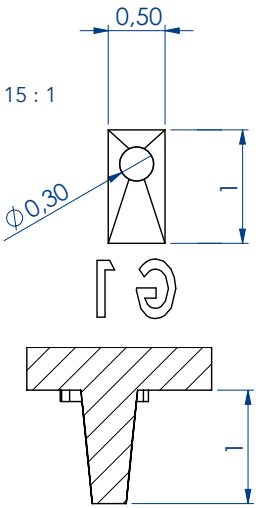


STAMPWELL **V**

V-shape 500 µm - for the imaging and culture of large-size rounded 3D objects such as spheroids and organoids

Number of pins: 42

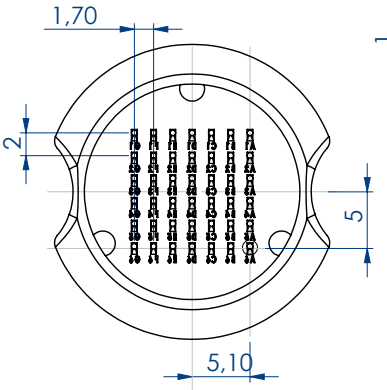
Pins imprint wells with the following dimensions:

- **Depth of wells:** 1 mm
- **Wells bottom diameter:** 500 µm - **Shape:** circular
- **Wells upper side:** 1 mm * 0,5 mm - **Shape:** rectangular
- **Spacing between 2 wells:** 1,70 mm on the lines and 2 mm on the columns

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

The right edge of the wells provides a slope to support and guide the pipette cone when loading the samples.

V-shape 500 µm



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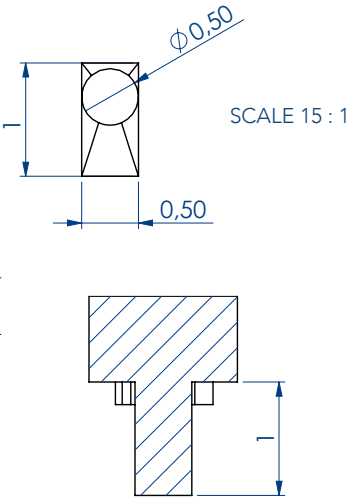


STAMPWELL Z

Embryo 1 - for the imaging of embryos such as 1 DPF zebrafish, Medaka or Astyanax embryos

Number of pins: 35

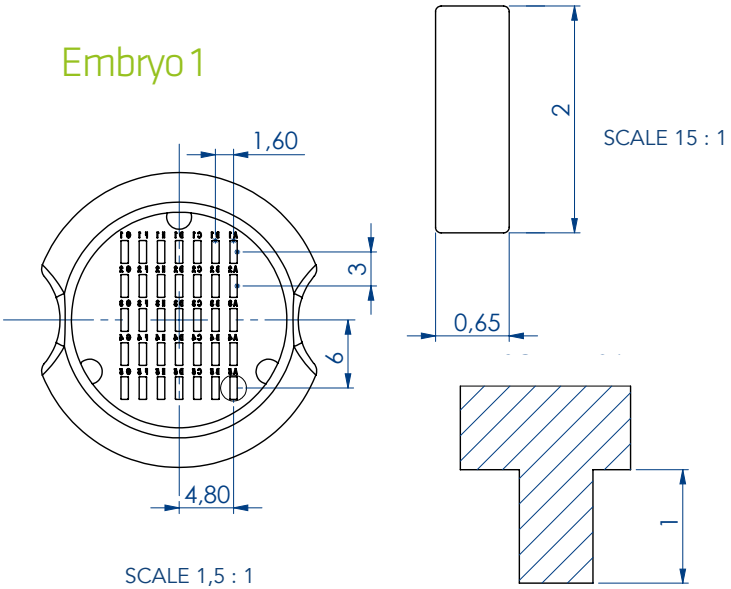
Pins imprint wells with the following dimensions:

- **Depth of wells:** 1 mm
- **Length * width:** 2 mm * 0,65 mm
- **Spacing between 2 wells:** 1,60 mm on the lines and 3 mm on the columns

Position of embryos in the wells: laying face downwards

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

Embryo 1



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STAMPWELL Z

Embryo 2 - for the imaging of 2 DPF zebrafish embryos or 9 DPF (stage 39) medaka embryos

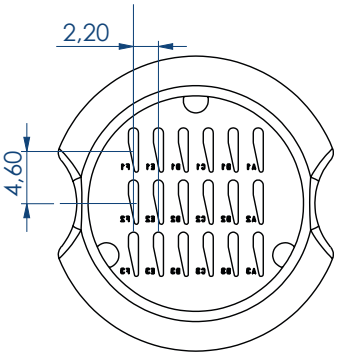
Number of pins: 18

Pins imprint wells with the following features:

- **Depht of wells:** 0.50 mm
- **Length of wells:** 3.90 mm
- **Width of wells:** 0.88 mm
- **Horizontal pitch:** 2.20 mm
- **Vertical pitch:** 4.6 mm

Position of embryos in the wells: laying on the side

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.



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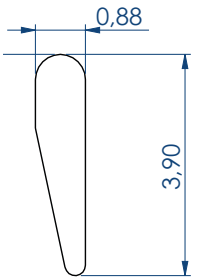
Embryo 2



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Stampwell | Specifications

STAMPWELL Z

Larvae 1 - for the imaging and culture of 3 – 14 DPF zebrafish or stage 39 - 41 medaka larvae

Number of pins: 15

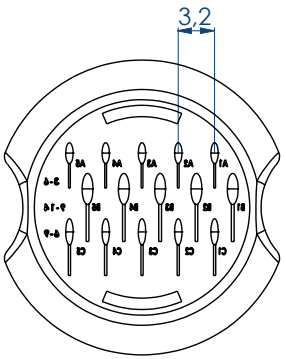
Pins imprint wells with the following features:

- **Wells shapes:** cuvette
- **Depth:** 0.6 – 1.4 mm
- **Dimensions of the smaller wells:** 4 x 0.65 mm
- **Dimensions of the intermediate wells:** 5 x 0.8 mm
- **Dimensions of the largest wells:** 6 x 0.96 mm

Position of larvaes in the wells: laying face downwards

Spotting: each well is identified with both a letter and a number. It allows the localization of the sample you want to further study.

Larvae 1



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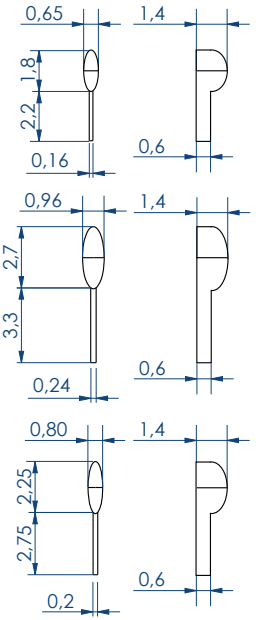

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STAMPWELL Z

Larvae 2 - for the imaging and culture of zebrafish or stage 39 - 42 medaka larvae imaging

Number of pins: 10

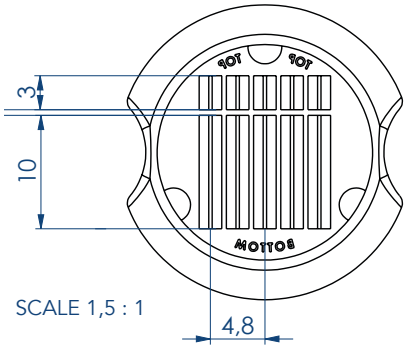
Pins imprint wells with the following features:

- **Wells shapes:** Prism
- **Depth:** 1 mm
- **Length for big wells:** 10 mm
- **Length for small wells:** 3 mm
- **Width of the bottom of the wells:** 0.3 mm
- **Width of the upper side of the wells:** 2 mm
- **Pitch:** 2.4 mm

Position of larvaes in the wells: laying face downwards

Spotting: simple coding (“top” / “bottom”)

Larvae 2



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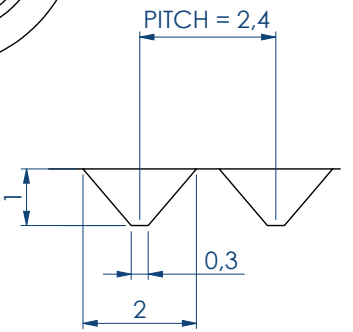

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Stampwell | Specifications

STAMPWELL **U**

U-shape - for cell aggregation into spheroids or organoids

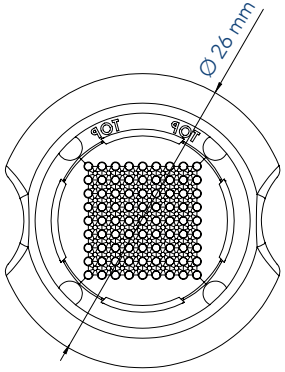
Number of pins: 81

Tips imprint wells with the following dimensions:

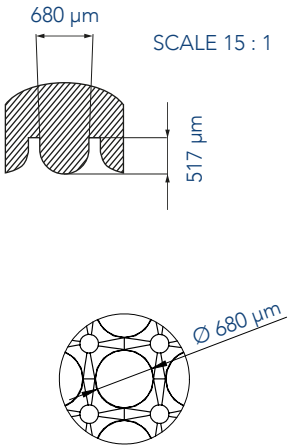
- **U-bottom**
- **Shape:** circular
- **Depth of wells:** 517 µm
- **Wells diameter:** 680 µm

Distance between the wells bottom and the dish bottom: 300 µm

U-shape



SCALE 1,5 : 1



SCALE 15 : 1

STAMPWELL U

STAMPWELL V

STAMPWELL Z

Check videos of protocol, examples
of results and much more on:
idylle-labs.com/shop

A protocol updated in May 2024

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