3D BIOPRINTING PROTOCOL

Claro™ BG800

Catalog numbers RGMB025 - 027 • Claro™ BG800 Bionk kit

Materials

- Claro™ BG800 Bionk kit (REGEMAT 3D, RGMB025 - 027)*
- 5 cc syringe (REGEMAT 3D, xxxx)*
- Blue syringe tips (details)
- Phosphate Buffer Saline (PBS)

Equipment

- Dual Tempered Syringe (REGEMAT 3D, XXXX)*
- UV light tool (450 nm, REGEMAT 3D, XXXX)
- BIO V1 or REG4Life 3D printer*
- Laminar flow cabinet*

Compatiblity with REGEMAT 3D's technology

✓ BIO V1 ✓ REG4Life

Protocol

1. Preparation and loading

1.1 Turn on laminar flow cabinet if sterile printing conditions are required. Let the air flow run for 5-10 minutes while cleaning the surface with EtOH 70% and preparing the required materials;
1.2 Turn on the printer, open the R3D Studio software and connect the printer using the power button on the top right of the screen;
1.3 Select the Platform menu > Petri dish.
Note: Remember Petri Dish should not be attached until after auto calibration.
1.4 From the Tools Selection menu, choose the print head (Tool 0 and T2) and the tools required (Syringe 5 cc > Dual Tempered Syringe; UV light > 450 nm, respectively).
Note: The dual tempered syringe tool only admits for its position to be in T0 and does not allow for any tool to be in T1 position.
1.5 Select the nozzle diameter and printing temperature. Click update tools to save the information.
1.6 Calibrate the required tools from the Tools Selection menu > Tool 0 > Autocalibrate and Tools Selection menu > Tool 2 > Autocalibrate.
Note: To perform autocalibration you can use an empty syringe so that the bioink is maintained at a constant temperature.
1.7 To perform the fine calibration, change the type of print bed to a Petri dish usignthe attatcment and click on Fine calibration to adjust the nozzle's height.
1.8 Pre-heat the print head to the desired temperature: 16 ºC.
Note: Printing temperatures vary according to GelMa concentration in the bioink, standarized concentrations and printing temperatures can be seen here. Mind specific temperature conditions inside the lab may vary printing temperature.

**Available on www.regemat3d.com*

1.9 Mix the cell pellet with the hydrogel
2.0 Once tool has reached the desired temperature, retrieve syringe from the fridge and place it inside the tool.
2.1 Leave the syringe inside the tool for 5 - 10 minutes so that the whole material is at the desired temperature.
Note: Extrude some material before printing to ensure material has de right texture and is at the tip of the nozzle.

2. Configuration and printing

2.1 Proceed to the tool configuration, by setting the parameters to the values indicated below:

Tool 0:

- Travel speed: 10.00 mm/s
- Change object speed: 6.00 mm/s
- Initial purge speed: 15.00 mm/s
- Initial purge volume: 3.00 µL
- Retract volume (travelling): 5.00 µL
- Retract volume (changing tool): 3.00 µL
- Retract speed: 50.00 mm/s
- Minimum travel time to retract: 0.30 s
- Compensate (travelling): 2.00 µL
- Compensate (changing tool): 10.00 µL
- Compensate speed: 50.00 µL/s
- W hop: 1.00 mm
- W hop speed: 15.00 mm/s
- Points deposition speed: 2.00 mm/s
- Flow: 1.50 µL/s
- Perimeter speed: 8.00 mm/s
- Infill speed: 6.00 mm/s
- Fill speed: 10.00 mm/s
- Skirt speed: 10.00 mm/s
- Support speed: 10.00 mm/s

Tool 2:

- Travel speed: 50 mm/s
- Change object speed: 30 mm/s
- Curing Speed: 1.10 mm/s

2.2 Load STL file or start the object configuration by pressing Load File > click on the blue arrow icon and select the desired geometry. The exact measurements can be configured next, in the Scaffold Configuration tab on the right-hand side;
2.3 Proceed to the scaffold configuration, by setting the parameters to the values indicated below:

- Scaffold height (H): 1.00 mm
- Scaffold diammeter (D): 10.00 mm
- Layer height: 0.25 mm
- Perimeter: 1
- Infill Type: lineal
- Pore size: 1.00 x 1.00 mm
- Initial angle: 0º
- Rotation angle: 90º
- Rotation every (layer): 1

2.4 Proceed to the skirt configuration (if required) from the tab on the right-hand side:

- Skirt distance: 3 mm
- Number of skirts: 1

2.5 Generate the print model by pressing on Slice on the top bar. This will generate a trajectory;
2.6 Start print.

3. Post-processing

3.1 Once printing is finished, close the Petri Dish and place it inside the laminar flow hood for 30 minutes.
3.2 After that time, soak the scaffold in PBS to help detach it from the bottom of the dish.
3.3 Using a spatula, the scaffold can be transferred to a 6-well plate

4. Results

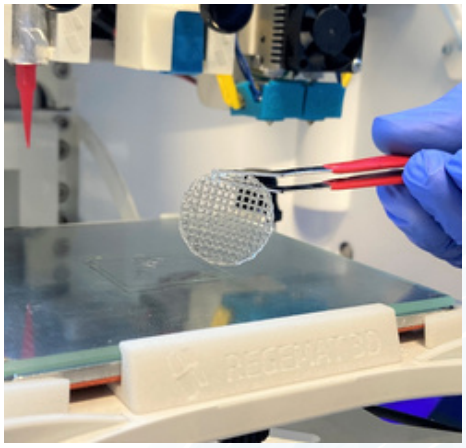

Figure 1. Macroscopic image of GelMA scaffold with cylindrical porous geometry. Scaffold from image was generated using the printing parameters from the previous section.

5. Troubleshooting