DYNAMIC MATRICES

DyNAtrix®
Minimal Discovery Kit

For Research Use Only. Not for use in human or animal therapeutic or diagnostic procedures.

Product Description

DyNAtrix is a fully synthetic, xeno-free cell culture matrix that enables precise adjustment of the mechanical cellular microenvironment. Due to its programmability and xeno-free composition, it allows highly reproducible cell culture studies under defined biochemical and mechanical conditions. The *Minimal Discovery Kit* provides four matrix formulations combining two matrix stiffness (G'= 50 or 100 Pa) and two different stress relaxation times (τ_{fast}~75 min or τ_{slow} ~1.1 days).

Materials

Materials included in the package*

B+	Backbone polymer with RGD peptides	800 µL
F1	Fast-relaxation crosslinker 1	200 µL
F2	Fast-relaxation crosslinker 2	200 µL
S1	Slow-relaxation crosslinker 1	200 µL
S2	Slow-relaxation crosslinker 2	200 µL

*all components are dissolved in 1x DMEM.

Storage and shelf-life

- Stored at 4 °C. Stable for at least 6 months. Do not freeze.

Additional equipment and materials required

- **Serum-free medium for the cells of interest.**
- **Cell culture ware:** compatible culture ware, e.g. multi-well plates, dishes.
- **(optional) Microwell inserts or plates:** recommended for small gel volumes, e.g. micro-inserts 4 well (ibidi GmbH, Cat. No. 80409), µ-Plate 96 Well 3D (ibidi GmbH, Cat. No. 89647) or similar.
- **Microcentrifuge tubes:** Eppendorf tubes or similar.
- **Ice bath:** for keeping gel components cold until use.
- **(optional) DNase I:** for gel degradation and cell release after 3D culture, e.g. DNase I (NEB, Cat. No. M0303).

Protocol

General remarks

- This protocol describes standard cell culture in dome or microwell format. The protocol can be adapted for single cells, small clusters, and organoids at different cell densities or culture formats.
- DyNAtrix is designed for use in fully defined media. **Do not add serum, KnockOut serum replacement, or conditioned media**, as they may degrade the matrix.
- For precise adjustment of matrix viscoelasticity, refer to Table 1.
- **Use matched crosslinker pairs only**: either [**B+**, **F1**, and **F2**] or [**B+**, **S1**, and **S2**]. Never combine crosslinkers that are labeled with different colors (e.g., do not combine **F1** and **S2**).

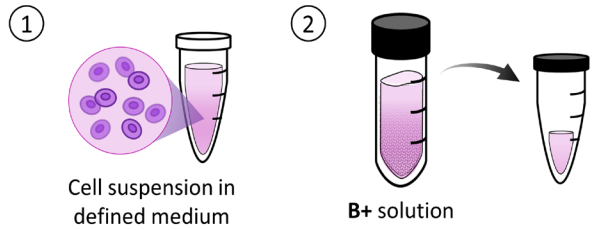
Preparation

1. Prepare a starting cell suspension at a density of 1–4 × 10^6 cells/mL in serum-free medium.

Dynamic Matrices i.G.
c/o Leibniz-Institut für Polymerforschung Dresden e. V.
Hohe Str. 6, 01069 Dresden
www.dynamicmatrices.eu | support@dynamicmatrices.eu

Note: If cells were cultured in a medium supplemented with serum, centrifuge cell suspension, e.g. at 300 ×g for 5 minutes, and resuspend the pellet in serum-free medium. Repeat the washing step once to remove serum residual completely.

2. Pipette 10 µL of **B+** into a sterile microcentrifuge tube. Keep it on ice.



Cell embedding at 4 °C / on ice

Select one matrix formulation before starting. Use **Table 1** to identify the required gel component volumes:

Table 1. *Matrix formulation for tuning viscoelasticity.*

Gel type	Target properties		Gel components (µL) per 25-µL mixture							
	Stiffness (G')	Stress-relaxation (τ)	B+	F1	F2	S1	S2	Serum-free medium	Cells	Total volume
1	100 Pa	75 min	10	5	5	-	-	-	5	25
2	100 Pa	1.1 days	10	-	-	5	5	-	5	25
3	50 Pa	75 min	10	2.5	2.5	-	-	5	5	25
4	50 Pa	1.1 days	10	-	-	2.5	2.5	5	5	25

*To scale up, multiply all component volume by the same factor.

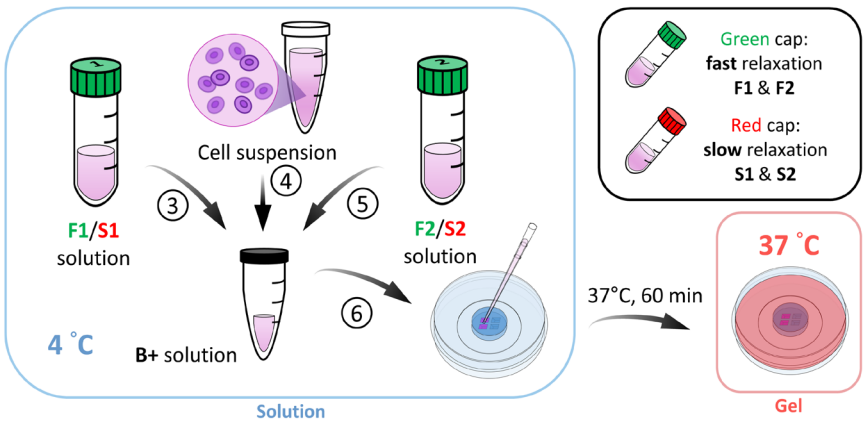
3. Add the first linker **F1** or **S1** to the 10 µL **B+** solution.
   Note: Mix thoroughly by pipetting up and down.

4. Add 5 µL cell suspension. For gel types 3–4, also add 5 µL serum-free medium or 1x DMEM.
   Note: Mix thoroughly by pipetting up and down.

5. Add the second linker **F2** or **S2** to the mixture.
   IMPORTANT: Mix thoroughly by pipetting up and down 80% of total volume (i.e. 20 µL) for ≥25 times for the best mixing quality. Keep the tip below the liquid surface to avoid bubbles.

6. Immediately dispense 5 µL per microwell or ≥20 µL per dome.
   Tip: Examine gel homogeneity under a microscope. Cells should be evenly distributed throughout the gel. If uneven distribution is observed, increase pipetting times in step 5.
   Note: The final cell density should be approximately 0.2–0.8 × 10^6 cells/mL.

7. Repeat steps 2–6 for preparing the next gel formulation.



Dynamic Matrices i.G.
c/o Leibniz-Institut für Polymerforschung Dresden e. V.
Hohe Str. 6, 01069 Dresden
www.dynamicmatrices.eu | support@dynamicmatrices.eu

Initiate culture conditions **at 37 °C**

8. Incubate at 37 °C, 5% CO_2 for **60 minutes** to allow complete gelation.
9. Gently cover the microwell insert or dome with serum-free medium.
10. Continue incubation at 37 °C, 5% CO_2 for the desired experimental duration.
    Note: Exchange the medium without directing liquid flow onto the gel to avoid gel detachment. Exchange frequency depends on the requirements of cell type, medium formulations and volume.

(Optional) Cell release from DyNAtrix **at 37 °C**

1. Add **DNase I** solution (2000 U/mL) to the medium. Recommended working concentration: 40 µL/mL (=80 U/mL).
   Tip: reduce medium volume before degradation to reduce required volume of DNase I.
2. Incubate at 37 °C, 5% CO_2 for **30–60 minutes**.
   Tip: Confirm cell release under a microscope. Cells should sediment down to the bottom of the plate. If not, add 0.5x volume more DNase I solution and incubate for another 30 minutes.
3. Collect the cells into a tube, centrifuge and resuspend the pellet in fresh medium or buffer for downstream analysis.
   Tip: If cells tend to stick to plate bottom, transfer the gels in advanced into a tube containing 0.2–0.5 mL medium before adding DNase I.

(Optional) Immunofluorescent staining in DyNAtrix **at RT / 4 °C**

This section describes a general staining protocol for mammalian cells.

1. Gently discard medium. Avoid disturbing the gels.
2. **Fixation:** Add 4% paraformaldehyde (PFA) and incubate samples at room temperature for 30 minutes.
3. **Washing:** Discard PFA. Add phosphate-buffered saline (PBS), incubate for 5 minutes at room temperature, and discard PBS. Wash for 3 times.
4. **Permeabilization and blocking:** Add permeabilization and blocking buffer (0.5% Triton™ X-100, 2% (w/v) bovine serum albumin (BSA) in PBS) and incubate for 30 minutes at room temperature.
5. **Primary antibody staining:** Add primary antibody at working dilution in 2% (w/v) BSA-PBS buffer and incubate overnight at 4 °C.
6. **Washing:** Discard antibody. Add PBS, incubate for 5 minutes at room temperature, and discard PBS. Wash for 3 times.
7. **Secondary antibody staining:** Add secondary antibody at working dilution in 2% (w/v) BSA-PBS buffer and incubate overnight at 4 °C. Protect samples from light.
8. **Washing:** Discard antibody. Add PBS, incubate for 5 minutes at room temperature, and discard PBS. Wash for 3 times.
   Tip: reduce non-specific binding of antibody by incubating in PBS overnight at 4 °C after the final washing step.
9. Immerse samples in PBS and store in the dark at 4 °C until imaging.
   Optional: Cover the gels with mounting media for long-term storage, e.g. ibidi Mounting Medium (ibidi, Cat. No. 50001).