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* allows dynamic adjustment of matrix stiffness (G'=1 to 100 Pa) and a choice of two different stress relaxation times (τ_{fast}~75 min or τ_{slow} ~1.1 days).

Materials

Materials included in the package*

B+	Backbone polymer +RGD	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.

Additional equipment and materials required

- **Culture dishes:** 6-well plates or 3.5 mm dishes for cell culture.
- **Microcentrifuge tubes:** Eppendorf tubes or similar.
- **ibidi inserts** (recommended for small culture volumes): micro-inserts 4 well for self-insertion (ibidi GmbH, Cat. No. 80409)

Protocol

General remarks

- The following protocol describes a standard cell culture experiment. Details of the protocol may be adapted to change parameters like cell density or total culture volume.
- DyNAtrix is designed for use in fully defined media. Avoid the use of serum or conditioned media, as this can lead to irreproducible results and matrix degradation.
- For precise adjustment of matrix viscoelasticity, refer to Table 1.
- A DyNAtrix gel contains either [**B+**, **F1**, and **F2**] or [**B+**, **S1**, and **S2**] but never combinations of crosslinkers that are labeled with different colors (e.g., do not combine **F1** and **S2**).

Table 1 DyNAtrix formulations for mechanical tuning.

Gel type	Mechanical properties		Gel components (µL)			
	Stiffness (G')	Stress-relaxation (τ)	B+	F1 / F2	S1 / S2	DMEM
1	100 Pa	75 min	10	5 / 5		
2	100 Pa	1.1 days	10		5 / 5	
3	50 Pa	75 min	10	2.5 / 2.5		5
4	50 Pa	1.1 days	10		2.5 / 2.5	5

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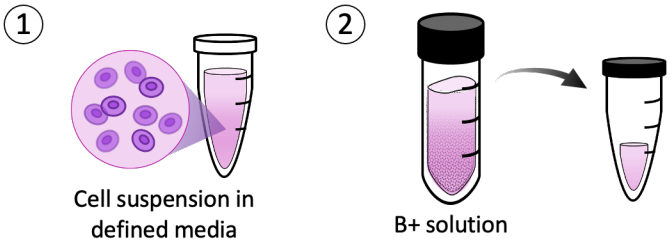
Preparations

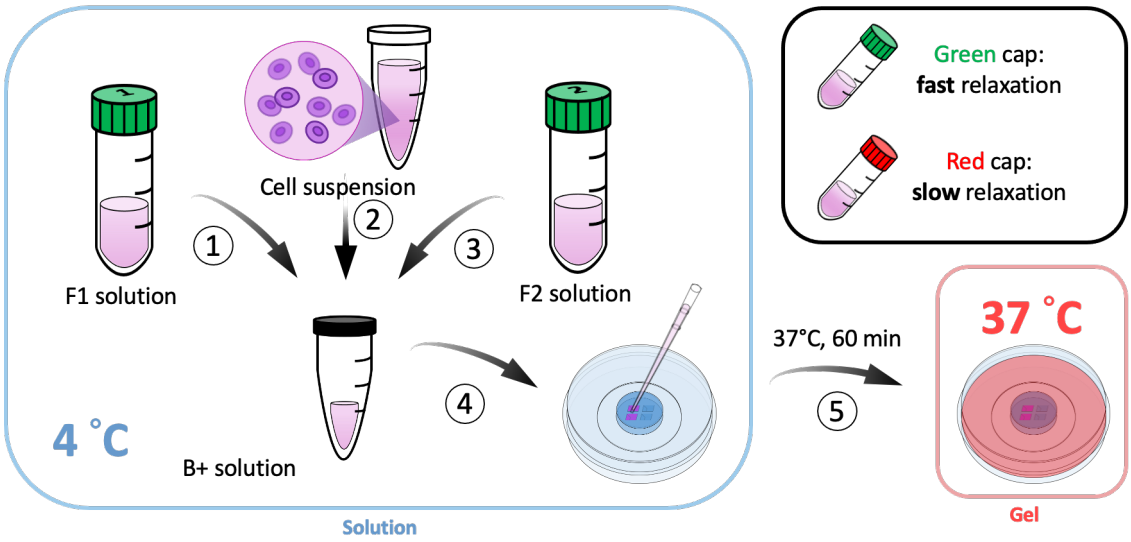
1. Prepare a cell suspension at a density of **4 × 10^6 cells/mL** in serum-free medium.
2. Add **10 µL** of **B+** into a sterile microcentrifuge tube for each insert to be prepared.
3. Place the ibidi inserts into 6-well plates or 3.5 mm dishes.



Cell embedding

Following this protocol will produce gel type 2 mentioned in table 1.

1. **Combine Crosslinker 1 with Backbone Polymer Solution**
   In an ice bath, mix **5 µL** of the **F1** or **S1** with **10 µL** of **B+ solution** in a sterile microcentrifuge tube.
2. **Combine Cells with Backbone Polymer Solution**
   In an ice bath, mix **5 µL** of the prepared cell suspension (4 × 10^6 cells/mL) with **15 µL** of the mixture obtained in Step 1.
   Note: Pipette up and down approximately **30 times** to ensure thorough mixing.
3. **Add Crosslinker 2**
   In an ice bath, add **5 µL** of **F2 or S2 solution** to the 20 µL cell suspension–polymer mixture.
   Note: Mix thoroughly by pipetting up and down approximately **30 times**.
4. **Dispense Gel into Inserts**
   Immediately inject **5 µL** of the mixture into each microwell, ensuring the gel surface is flat and uniform.
   Note: Examine gel homogeneity under a microscope. Cells should be evenly distributed throughout the gel. If uneven distribution is observed, increase pipetting during the mixing steps.
5. **Polymerization**
   Incubate at **37 °C, 5% CO_2** for **60 minutes** to allow complete gelation.
6. **Medium Overlay**
   Carefully overlay the entire insert with culture medium.
   Note: Avoid introducing air bubbles between the gel surface and the medium.
7. **Culture**
   Continue incubation at **37 °C, 5% CO_2** for the desired experimental duration.



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