# DyNAtrix®

# Troubleshooting Guide

**Ready-to-Use Kit (#DM-RUK-1) and Minimal Discovery Kit (#DM-MIDK-1)** | Version 1.1

## 1. Before cell embedding

| Issue | Possible causes | Recommendations |
|---|---|---|
| Component is yellow or turbid | • Possible microbial contamination.<br>• pH change caused by improper storage. | • Do not use the component; contact support with the lot number and photographs. |
| Component is unusually viscous, partially gelled, or difficult to pipette | • Cross-contamination between components initiated partial crosslinking. | • Use a new sterile tip for each stock vial.<br>• Contact support if the stock vials are partially gelled. |

## 2. During cell embedding

| Issue | Possible causes | Recommendations |
|---|---|---|
| Matrix remains liquid or does not gel after incubation for 60 min at 37 °C | • The final crosslinker was omitted, incorrect, or mismatched.<br>• Serum or conditioned medium was carried into the matrix. | • Verify component volume and the matched crosslinker pair for the Minimal Discovery Kit.<br>• Wash cells twice in serum-free medium before embedding to minimize serum carryover. |
| Gelation happens before dispensing | • Components or the mixture warmed before dispensing.<br>• Final crosslinker was added to the mixture and sat too long before dispensing. | • Keep components and the mixture cold at 4 °C or on ice during preparation.<br>• Add the final crosslinker immediately before dispensing. |
| Bubbles are introduced during mixing or dispensing | • Mixing was too rapid, or the full volume was expelled forcefully with air mixed into the mixture. | • Keep the tip below the liquid surface and pipette about 80% of the total volume.<br>• Quickly spin the tube on a mini benchtop centrifuge to remove bubbles.<br>• Remove a surface bubble carefully after dispensing with a sterile tip without disturbing the gel. |
| Cell-matrix mixture is too viscous or difficult to pipette | • B+ is slightly viscous by design.<br>• Partial gelation has started. | • Use a positive displacement pipette to mix and dispense for accurate volume and low material retention in tips. |
| Cells are unevenly distributed within gels | • Incomplete mixing.<br>• The cell stock settled or contained clumps before the aliquot was taken. | • Mix by pipetting up and down 20 µL for 25 times for homogenous mixing; avoid bubbles.<br>• Gently resuspend the cell stock before taking the aliquot. |
| Spheroids or organoids look fragmented after mixing or dispensing | • The pipette-tip opening was too narrow, causing excessive shear.<br>• Spheroids were too large. | • Use a wide-bore tip for mixing and dispensing.<br>• Prepare spheroids no larger than 100 µm in diameter before embedding to reduce shear stress. |
| Mixture looks inhomogeneous or phase-separated | • Incomplete mixing, or an incorrect order of addition. | • Prepare a fresh sample using the exact volumes and order; mix thoroughly by pipetting up and down for ≥25 times. |

## 3. After cell embedding & in culture

| Issue | Possible causes | Recommendations |
|---|---|---|
| Gel dome detaches or is lost during medium handling | • Medium was added or removed directly onto the gel. | • Add and aspirate medium along the vessel wall. Use a microwell insert for small gels or drop a larger dome volume. |

| Issue | Possible causes | Recommendations |
|---|---|---|
| Dome breaks or collapses | • Incomplete mixing or gelation. | • Prepare a fresh sample using the exact component volumes and order; mix thoroughly by pipetting up and down for ≥25 times.<br>• Incubate the mixture at 37 °C for 60 min to allow complete gelation. |
| Gel degrades unintentionally, and cells sediment to the bottom | • Serum, KnockOut Serum Replacement, conditioned medium, or another nuclease-containing supplement was used.<br>• Serum or conditioned medium was carried in with the cells.<br>• Extensive cell death released nucleases. | • Use fully defined, serum-free medium throughout the culture unless a formulation has been specifically validated.<br>• Wash cells twice in serum-free medium before embedding.<br>• Contact support with the medium composition if degradation persists. |
| Spheroids or organoids sediment to the bottom during gelation | • Large or dense aggregates weight heavier and sediment before gelation is complete. | • Flip the plate upside down for the first 10 min, then return upright for the rest 50 min of incubation at 37 °C. |
| Little or no cell proliferation or organoid expansion | • The defined medium lacks cell-type-specific growth factors or nutrients.<br>• The RGD ligand in the matrix is insufficient for the model. | • Run a cell-only control on 2D or in suspension to confirm viability in the serum-free medium.<br>• Supplement B+ with 0.2-0.5 mg/mL (recombinant) laminin before gelation.<br>• Contact support to discuss alternative adhesion ligands. |
| Unhealthy morphology or cell death | • Excessive pipetting shear occurred during mixing or dispensing.<br>• The defined medium is not optimal for the cell model.<br>• The RGD ligand in the matrix is insufficient for the model. | • Use wide-bore tips for cell embedding.<br>• Contact support for serum-free medium suggestions.<br>• Contact support to discuss alternative adhesion ligands. |

## 4. Cell release from DyNAtrix

| Issue | Possible causes | Recommendations |
|---|---|---|
| Gel degradation is slow or incomplete after DNase I treatment | • DNase I concentration or activity was insufficient. | • Add more DNase I to the medium or extend incubation time. Use fresh, active DNase I for optimal performance.<br>• Gently pipette the gels to mechanically break them and facilitate degradation. |
| Released cells adhere to the vessel before collection | • The gel was digested in a tissue-culture-treated vessel that promotes cell adhesion. | • Transfer the gel to a low-binding tube containing 0.2-0.5 mL medium before adding DNase I.<br>• Pre-wet the tube and tip with 1% BSA solution to cover the surface with anti-sticking coating. |
| Residual gel or DNase I interferes with downstream analysis | • Incomplete gel degradation or insufficient washing. | • Wash released cells by centrifuging and resuspending the pellet in fresh, assay-compatible buffer or serum-free medium. Verify that visible gel fragments are gone. |

## 5. Immunofluorescent staining in DyNAtrix

| Issue | Possible causes | Recommendations |
|---|---|---|
| DAPI or Hoechst nuclear signal is weak in cells or the matrix background is strong | • DyNAtrix contains DNA-based crosslinks that can be stained by DNA-binding nuclear dyes. | • Increase dye concentration for ≥5 folds.<br>• Release the cells before staining. |
| Antibody signal is weak or visible only near the gel surface | • Antibody penetration is slow in thick 3D samples. | • Extend antibody incubation time and gently rotate the plate while keeping the gel fully immersed.<br>• Use Fab fragments or nanobodies instead of full-length antibodies for faster penetration.<br>• Use a smaller or thinner gel format.<br>• Release the cells before staining. |
| High nonspecific binding of antibody in matrix background | • Washing was insufficient.<br>• Unbound antibody did not diffuse out of the gel. | • Incubate in the washing solution (1%BSA in PBS) overnight after primary antibody staining to remove unbound antibody. Gently rotate the plate while keeping the gel fully immersed.<br>• Repeat extended washing by incubating in the washing solution overnight after secondary-antibody staining.<br>• Use Fab fragments or nanobodies instead of full-length antibodies for faster diffusion. |
| Gel detaches or floats during imaging | • The culture vessel does not retain the sample during repeated washing. | • Remove most buffer and cover the floating gel with a coverslip to stabilize its position. Do not allow the gel to dry. |
| Deep structures are blurred or difficult to image | • Light scattering and refractive-index mismatch in a thick sample.<br>• The culture vessel is not glass-bottom. | • Immerse the gel in mounting media with a refractive index of 1.42-1.45 to minimize light scattering.<br>• Perform optical clearing in DyNAtrix after fixation, such as delipidation in SDS buffer, to increase transparency of large multicellular structures.<br>• Use an imaging-grade vessel with a #1.5 glass bottom or equivalent. |

## 6. Information to provide when contacting technical support

- ☐ Kit name and lot number.
- ☐ Cell type and medium type.
- ☐ Culture vessel type.
- ☐ Culture duration.
- ☐ Dyes, antibodies, and staining methods, if relevant.
- ☐ If available, provide photograph or microscopy images to illustrate the issue.