Dissolution of Dextran-Based Hydrogels with *3-D Life* Dextranase - Recovery of Cells

1. Introductory Notes

- Live cells can be recovered from dextran-based *3-D Life* Hydrogels by using *3-D Life* Dextranase. The dextranase cleaves the glycosidic bonds of dextran and thus induces the degradation of the gel.
- After dissolution of the gel, repeated washing of the cells is important to remove residual gel constituents and dextranase from the cell suspension to avoid unwanted interference of these compounds
  - with subsequent analytic measurements (e. g. Western blotting or other analyses),
  - and with subsequent cultivation of the recovered cells in a fresh dextran-based hydrogel. Traces of *3-D Life* Dextranase could cause a destabilization of the newly set hydrogel.
- As the dissolution of the hydrogel by *3-D Life* Dextranase is an eroding process, degradation of the gel occurs from the surface. The degradation of large gels can be accelerated by cutting the gels in pieces.

2. Protocol

Reagents and materials:

***3-D Life* products:**

3-D Life Dextranase (Catalog Number D10-1)

Additional reagents and materials:

Pipet tips, micropipets, serological pipets, 15 mL centrifuge tubes, cell culture medium or PBS, ice.

Experimental procedure:

The following protocol is intended for a 10-30 µl dextran-based hydrogel cultured in 500 µl growth medium. It can be scaled up or down as required. However, a change in the volume to surface ratio of the hydrogel may change the required time for degradation.

If not indicated otherwise all steps below are performed in a sterile hood.

1. Add 26.3 µl *3-D Life* Dextranase to the cell culture supernatant for a 1:20 dilution of dextranase.
2. Incubate at 37°C in the incubator for 30 minutes.
3. Check the degradation of the gel by aspirating the medium with a 1000 µl pipet tip. After inspection, release the medium back to the well.
4. If not fully degraded, continue incubation at 37°C until the gel is completely dissolved.
5. Once the gel is fully degraded, resuspend the cells and place the cell suspension in a 15 mL centrifuge tube.
6. Add 10 mL of cold medium or physiological washing buffer (e. g. PBS).
7. Pipet the suspension two times up and down.

8. Sediment cells by centrifugation at 180 x g for 5 min at 8°C, or as compatible with your cells.
9. Carefully aspirate the supernatant down to the pellet.
10. Repeat step 6-9 two times.
11. Resuspend cells for your downstream cell analysis or culture, as appropriate.
12. Keep cells on ice until further use.

Troubleshooting

Observation	Possible causes	Possible remedies
Dissolution of dextran-based hydrogel with *3-D Life* Dextranase is not complete after incubation for 1 hr at 37°C.	High gel strength; large gel volume to surface ratio.	Incubate gel up to 2 hr; cut the gel to pieces prior to digestion; increase *3-D Life* Dextranase concentration up to a 1:10 dilution.
	3-D Life Dextranase activity reduced due to long storage at 4°C (>6 months) or higher.	Use new aliquot of *3-D Life* Dextranase and adhere to storage conditions indicated in the Product Data Sheet.
Cells die during dextranase digestion.	Cells are sensitive to the *3-D Life* Dextranase solution.	Reduce *3-D Life* Dextranase concentration to a dilution of 1:30 or 1:40. If the digestion is too slow, cut the gel to pieces.
After seeding cells in a fresh *3-D Life* Dextran Hydrogel the gel starts to become unstable and dissolves gradually (this may take up to a few days of cultivation).	Traces of *3-D Life* Dextranase were carried over into the freshly set gel.	Wash cells more than 3 times after dextranase digestion to remove residual dextranase; use less dextranase for gel digestion, e. g. a 1:30 or 1:40 dilution or less; include 10 mg/mL dextran 6 (Carl Roth, Cat. No. 7619.2) in the culture medium of the new set gel to prevent dissolution.

FAQs

Q: Can I degrade the gel also in PBS?

A: Yes, but longer incubation times or higher Dextranase concentrations may be needed.

Q: Does the dissolution of gels also work after chemical fixation?

A: Yes. If cells have been fixed with paraformaldehyde (e. g. 4% in PBS, one hour), the gel can be degraded after extensive washing of gels with PBS (e. g. four exchanges of PBS including an overnight incubation). Then add a 1:20 dilution of dextranase in PBS and continue with gel degradation as described above for live cells (step 2.). Other chemical fixations have not been tested yet for their compatibility of dextranase-mediated gel degradation.