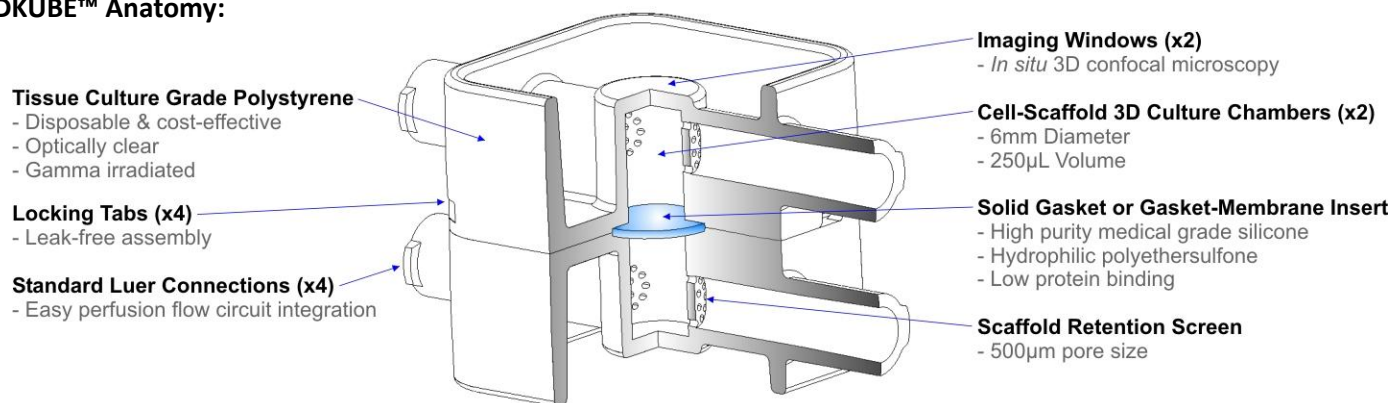


3DKUBE™ 3D Cell Culture Plasticware

Store at Room Temperature

Model Number	3DKUBE™ Configuration	Included Gasket Component	# of Samples per 3DKUBE™
3D010	Segregated Co-Culture	Silicone Gasket-PES Membrane Insert (0.45 μm pore size)	n=1
3D020	Independent Chambers	Solid Silicone Gasket	n=2
3D030	Cell Migration	Silicone Gasket-PES Membrane Insert (3.0 μm pore size)	n=1

3DKUBE™ Anatomy:



Preparation for 3DKUBE™ Custom Experiments:

- Obtain the 3DKUBE™ configuration appropriate for your desired experimental protocol.
- Gather any additional materials for a perfusion system and sterilize appropriately. A list of [Suggested Parts and Assembly](http://www.kiyatec.com/product.html) can be found by visiting www.kiyatec.com/product.html.
- Based on the desired cell type(s) and scaffold material(s) for your custom experiment, determine the preferred cell seeding method:
 - Cell Encapsulated Scaffold*: Pre-seed scaffold material before loading into culture chamber.
 - Perfusion of Scaffold with Cell Injection*: After loading culture chamber with acellular scaffold material and assembling the system, inject a cell suspension through a 4-way stopcock valve to seed the scaffold *in situ*.
- Perform all procedures aseptically in a laminar flow hood for optimal results.

Loading & Assembly of 3DKUBE™ Chambers:

- Peel back the 3DKUBE™ Tyvek® lid. The cell culture chambers will remain in the loading tray.
- Using sterile tools, place desired scaffold material (pre-seeded or acellular) into one chamber.
- Using sterile forceps, place the silicone surface of the gasket insert within the recessed groove over the scaffold-filled culture chamber. Gently press around the circumference of the gasket to ensure static adhesion.
- Using sterile tools, place desired scaffold material in the remaining chamber.
- Carefully take up one half of the 3DKUBE™ in each hand.
- Invert** the culture chamber with the gasket insert, **align** the opposing faces of the two culture chambers to create a small angular offset of the corner locking tabs, apply slight pressure and **twist** clockwise to **click** the locking tabs in place.

3DKUBE™ Workflow

- Open 3DKUBE™ Tray
- Load Chambers with 3D Scaffold of Choice
- Assemble 3DKUBE™
- Attach 3DKUBE™ into Perfusion System and Incubate
- Analyze 3D Culture Using *in situ* Techniques

Assembly of Perfusion System:

- Assemble perfusion flow circuit using luer connections, tubing, and medium reservoir of choice to meet specific requirements of your experiment. A suggested assembly can be found by visiting www.kiyatec.com/product.html.
- Clip the tubing portion of the assembly into the peristaltic pump cartridge or connect to syringe pump.
- Fill medium reservoirs and adjust to the desired flow rate. Internal KIYATEC research typically uses 0.1-2.0 mL/min.
- Cell Injection Seeding Method*: Stop fluid flow, adjust the 4-way stopcock valve for seeding at the inlet of the culture chamber, inject a cell suspension through the 4-way stopcock and allow the cells to attach under static culture conditions for approximately 1 hour. After cell adhesion, reset the 4-way stopcock valve and commence dynamic perfusion flow.