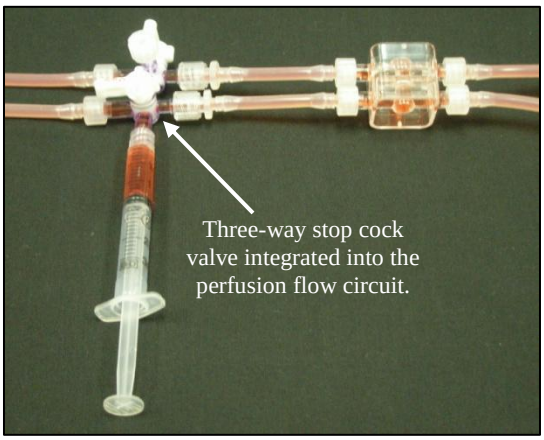
3DKUBE™ *In Situ* Biochemical Assays

Standard biochemical assays can easily be adapted for use with 3DKUBE™ 3D Cell Culture Plasticware. Using a three-way stopcock valve integrated into the flow circuit, aliquots of medium can be taken for use in cell function and characterization assays. Such assays include but are not limited to MTT, lactate content, glucose content and albumin content. Additionally, metabolic assays such as alamarBlue® can be performed *in situ*…no need to disturb the 3D cell culture environment!

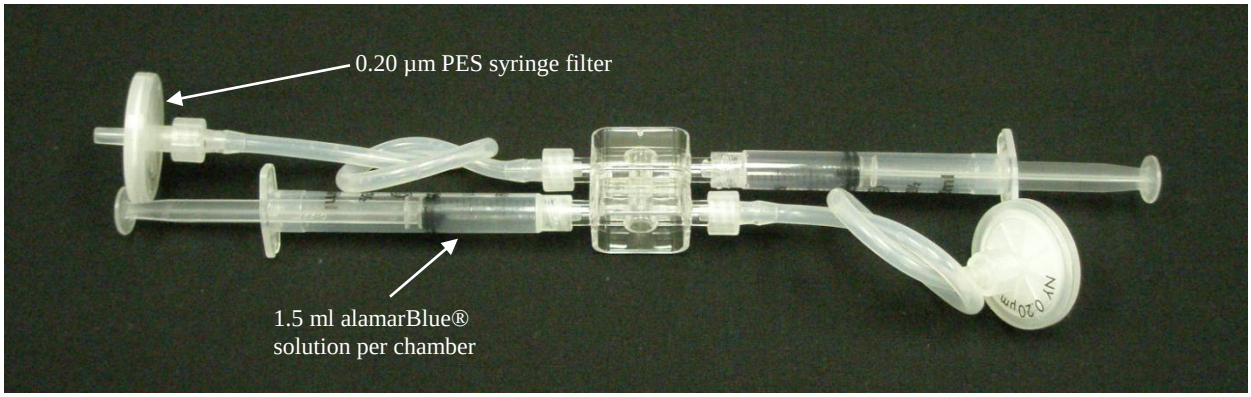
3DKUBE Medium Sampling:

1. During experimental setup, incorporate a sterile three-way stopcock valve (with male plug) within the perfusion flow circuit.
2. At desired time points using aseptic technique, stop the perfusion flow, attach a 3 mL syringe to the three-way valve, position valve and remove a desired volume of medium for sampling. Return stopcock valve to original perfusion position.
3. Return 3DKUBE and perfusion circuit to cell culture environment until next sampling time point.
4. Continue with desired biochemical assay procedures.



3DKUBE™ *In Situ* alamarBlue® Assay:

1. Prepare alamarBlue® solution according to manufacturer's guidelines.
2. Fill two 3 mL syringes, one for each chamber, with 1.5 mL of alamarBlue® solution and attach to opposing sides of the 3DKUBE (shown below).
3. Attach a short length of sterile silicone tubing and 0.20 µm PES syringe filter with standard luer connections to the 3DKUBE's female luer connections opposite of the syringes (shown below).
4. Inject approximately 400 µL of alamarBlue® solution every 15 minutes for 1 hour, incubating at 37°C between injections. This can be accomplished manually or with a syringe pump.
5. Withdraw approximately 400 µL of alamarBlue® solution back into the syringe every 15 minutes for 1 hour, incubating at 37°C between withdrawals.
6. Following injection and withdrawal procedures (4 & 5), withdraw any remaining alamarBlue® solution from each chamber and remove the syringes. Aseptic technique during this process will permit ongoing culture of the cell population contained in the 3DKUBE.
7. Measure the resulting alamarBlue® fluorescence by injecting the metabolized solution (contained in the syringes) into a 96-well plate according to the manufacturer's guidelines.



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