FAQ Questions from Family and Friends Webinar (1-27-2022)

Question: *Have they ever been used for iPSC culture maintenance and/or differentiation of iPSCs to specific cell types?*
Answer: Yes, the T-Blocks have been used to culture and maintain iPSCs derived from human skin cells. The T-Blocks were coated with vitronectin. However, no experiments to our knowledge have been performed that differentiate iPSCs in the T-Block. These are experiments we are excited to explore with collaborators to better customize the T-Blocks for iPSC culture and differentiation.

Question: *Can you talk about non adherent cells?*
The remarkable thing about this platform is its agnostic. It should work with any cell line in any situation. And because we eliminate subcultures, reduce senescence, and get more consistent phenotype this should all mean more consistency in all cultures. We have not tried every cell line. What we might suggest, let's open a dialog about what you are trying to achieve, get you some Bio-Blocks, and collaborate to increase success potential.

Question: *Dr. Mellott mentioned you may add an X-Block to a T-Block, later take that away for extraction and keep the culture in the original T-Block. How long can you do that, i.e., keep a culture in a T-Block as a "starter" culture to grow into adjacent blocks? Is there natural degradation of the T-Block?*
Answer: Technically indefinitely, or until your block becomes confluent which will vary by cell line. By adding a second/third/fourth/etc. you elongate the life of the first block. This is due to cells having room to migrate to the new block. As for degradation, we are sure that exists at some point. But we have noticed zero degradation up to six months.

Question: *How do you use the blocks with Roller Bottles on rolling racks?*
Answer: First use a non-adherent roller bottle for best results. Then add a little media, to prevent a block from accidently sticking to the bottle. Seed your blocks in a standard non-adherent plate or dish. Then place the block inside the roller bottle and add media. Media usage will vary. We recommend 8.5 mL per block. But if you only use 1 block, you will need more media to cover the side of the bottle as it rotates. If you use more blocks you will have to utilize media accordingly.

Question: *Are blocks already sterilized or need to be autoclaved? irradiation?*
Answer: The blocks come sterilized. Please do not autoclave

Question: *How many T-Blocks do I need to replace 850 cm^2 RB?*
Answer: If you goal is just cell production 1 Bio-Block can produce as many cells as 10.6 x 850 cm^2 roller bottles. This answer will vary depending on length of incubation and cell line. If you are interested in biological by-products the answer will be similar, but we will need more details to answer that correctly. And the best answer is for us to support you in getting blocks and working through the solution.

Question: *Have you used T blocks with chicken embryo fibroblastic cells?*
Answer: We have worked with fibroblasts but not specifically chicken embryos; although we would be glad to collaborate with you to achieve that.

Question: *Specific gravity of the T-Block? Will it float?*
Answer: Yes, they will float slightly. This will depend a little bit on the amount and density of your media. You can insert an air bubble in the block using a pipette that makes it float more.

Question: *Have you tested for genetic drift head-to-head (i.e., T75 vs Ronawk block)? It seems like your technique would allow for a lower passage number and less mutation. Definitive evidence of this would be a very critical piece of data.*
Answer: Yes, we have, and you are correct. There is only ever one passage. There is significantly less genetic drift. And there is significantly less mutation. Answers to this question will vary by cell line and the best test is for you to start using the blocks and optimize for your research/process. Also consider we have not tested every cell line. For best results try the Bio-Blocks and how they apply to your specific research.

Question: *Can imaging be performed on the blocks?*
Answer: Yes. See this link here taken on a confocal. https://youtu.be/HnBE8tPeI0w

Question: *Can you stack in z-direction, e.g., create a combined block of 2x2x2 cm³?*
Answer: Not with the current blocks we offer, but in the future, we plan to enable blocks to be stacked in the Z-direction.

Question: *Nanovesicles/ exosomes production can be done using bio blocks.*
Answer: Yes.

Question: *Can we look at the T-blocks under the microscope like you would say a T300 flask? Or is there a better way to image cell growth progress?*
Answer: Yes, you can look at the blocks through a flask on most microscopes. But due to the neck of flasks it is sometimes hard to get the blocks out. We recommend plates, dishes, or a product like TPP's re-closable flask over the traditional T25/T75/etc.

Question: *Is there any sort of early indicator if there's a blockage in the microchannels (other than media pH)?*
Answer: Yes, when you pipette liquid through, and it pour out the top there is blockage. The liquid should travel through the block like a sieve.

Question: *Can you grow lymphocyte, macrophages, or dendritic cells in T-block? If yes, for how long?*
Answer: There is no reason you should not be able to grow lymphocytes, macrophages, or dendritic cells. We have not grown any of these cell types yet but are excited to collaborate with you on growing these cell types.

Question: *How do you change media with different small molecules growth factors at different stages of cell differentiation?*
Answer: Just like you would with any cell culture. You just aspirate media from the side of the well, and if you need to clear out media from the inside one of the blocks, simply flush PBS through the block by gently pipetting from the top of the block. Aspirate the residual PBS and add fresh media to the side of the well and submerge the block.

Question: *Is there a concern for Mycoplasma?*
Answer: No. The Blocks come completely sterilized.

Question: *Does the same apply for endotoxins?*
Answer: We believe this is a follow up to the mycoplasma question. If that is the case, then there are no endotoxins in the Blocks. If not, please reach out so we can better understand your concerns.

Question: *How do you monitor contamination or lack thereof?*
Answer: By monitoring the media pH, the surfaces of the Block, and testing the spent media for mycoplasma.

Question: *How are cells harvested?*
Answer: We have an enzymatic reagent, called "X-Tract" that can be added to our X-Blocks to degrade them to release over 90% of cells. Cells can be harvested from the E and T-Blocks using X-Tract as well, but not to the efficiency of X-Blocks. For best results, use X-Tract to harvest cells from all our blocks.

Question: *Can cells be characterized in the blocks?*
Answer: Cells inside the T-Blocks can be characterized by processing like tissue: Fixed, embedded, sectioned, and stained. The T-Block is ideal for cells that need a coating to grow and is the sturdiest of blocks. Basic cell dyes can be used on the X-Block and the E-Block for visualizing cell nuclei, cell membranes, and cell organoids. However, the T-Blocks is best for histological and antibody labeling.

Question: *Are the blocks GMP grade?*
Answer: Not yet, but we are in the process of developing our own GMP Facility. In the meantime, we are delighted to collaborate with you on evaluating the Bio-Blocks in your applications so that they are ready to use when we can produce them under GMP conditions.

And we would like to end with this statement from a customer: "***Thank you so much for that delightful presentation about your novel products. It was also a pleasure to see some results related to our collaboration, a study about the generation of spheroids on T-blocks. We are a group that is excited about the idea behind the Bio-Blocks and wish you the best of success. Congrats to the team on your newest product, X-Block!"***