### Frequently asked questions

#### How are spheroids differ from organoids?

Organoids are complex 3D cell clusters including organ-specific cell types. These cell types are derived from stem cells, progenitor cells or iPSCs which have the capacity to differentiate to different lineages of tissues and self-assemble into organoids with an in vivo similar architecture. To maintain organoid culture optimized cell culture condition the addition of e.g specific factors and extra cellular matrix components are needed.

Spheroids are simple, self-aggregated 3D cell clusters containing one or more cell types (primary cells or immortalized cell lines) of certain tissue components. Spheroids recapitulate tumor microenvironment due to their architecture which includes a necrotic core, quiescent and proliferating cells. Further spheroids have a high potential for applications in drug discovery.

#### <u>Are spheroids not scaffold-based systems? Is it a scaffold-based system if</u> proteins are added to improve spheroid formation?

Scaffold-free systems such as spheroids in low-adhesion plates rely on the formation aggregates by self-assembly. The 3D structure is hold by cell-cell contacts between the cells. In contrast, in scaffold-based systems the cells are embedded in a network of synthetic or natural components. These materials interact with the cells, support cell adhesion and are necessary to maintain the 3D structure. The addition of proteins into the media which support the spheroid formation via cell-cell contacts is in our definition not scaffold-based because these proteins are not acting as a bearing framework/scaffold.

#### Why use BIOFLOAT<sup>™</sup> coating technology for your research?

BIOFLOAT<sup>™</sup> coating technology provides a highly defined, fully inert cell- and protein-repellent surface for cell culture labware and devices. Compact and reproducible spheroids are rapidly generated on BIOFLOAT<sup>™</sup> coated surfaces, even with challenging cell types. Sticky proteins are inhibited to adsorb to your cell culture labware and thereby prevent cell adhesion.

#### How spheroids are formed in Biofloat<sup>™</sup> plates?

In Biofloat<sup>™</sup> plates cells are not able to adhere to the cell- and protein repellent surface. Therefore, cells remain in suspension, get into contact with each other and form a dense cell network.

#### Which cells are compatible with the BIOFLOAT<sup>™</sup> coating technology?

BIOFLOAT<sup>™</sup> coating technology is compatible with broad range of human and animal derived cell lines or primary cells. This new technology is developed to generate cell spheroids; therefore, any cell line can be used which are known to form such spheroids or you can develop new organoid models containing spheroids with cell lines of your choice. In Tab. 1 you can find a selected number of cell lines cultured with BIOFLOAT<sup>™</sup> coating technology.

#### Tab. 1 List of cell lines

Cell line		
3T3 (Mouse fiboblasts)		
A431 (Epidermoid carcinoma cell lines)		
Capan-1 (Human pancreatic adenocarcinoma cell Line )		
CHO (Chinese hamster ovarian)		
D492 (Breast epithelial stem cell like lines)		
D492HER (Tumorigenic breast epithelial stem cell of D492)		
DAN-G (Human pancreas carcinoma cell line)		

FAMPAC (Human pancreatic adenocarcinoma cell line) Fibroblast precurser (Rainbow trout) H1975 (Human Lung adenocarcinoma) H2228 (Human Lung adenocarcinoma) H3122 (Human Lung adenocarcinoma) HCC1433 (breast cancer cell line) hDPSC (Primary human dental pulp stem cells hDPSC+Panc1 (Human pancreatic cancer cell line) HEK293 (Human embryonic kidney cell line) HepG2 (Human hepatoma cell line) huARLT(Immortalized endothelial cells originating from HUVEC cells) HuOB (immortalized human osteoblast) huVEC (Human vein endothelial cells) iPSC-Gata6 (induced pluripotent stem cells) MCF10A (Breast cancer cell lines) MCF-7 (Breast cancer cell line) MDA-MB231 (Breast cancer cell lines) Mia-Paca (Human pancreatic cell line) Neuronal stem cells (HN9 differentiated) Panc1 (Human pancreatic cell line) Panc39 (Human pancreatic cell line) PRH with RHSteC (Rat hepatic stellate cell) PRH+ HHSteC (Human hepatic stellate cells) Primary hepatocytes (Human, Dog, Monkey, Mouse) RPMI (B-lymphocytes myeloma cell lines) SFFV2 Human immortalized astrocytes

#### Which products are available with the BIOFLOAT<sup>™</sup> coating technology?

Choose between the ready-to-use 96-well plate or DIY BIOFLOAT<sup>™</sup> FLEX coating solution to modify a broad range of plastic and glass surfaces.

#### What are the applications of BIOFLOAT<sup>™</sup> FLEX coating solution?

The BIOFLOAT<sup>™</sup> FLEX coating can be easily used to create a homogeneous and robust coating for your cell culture products, which is stable under standard culture conditions. One pipetting or rinsing step is enough to passivate your surface on the nanometer scale without altering the geometry of your device. The coating solution allows to treat different formats of plates, microfluidic devices or bioreactors, which are amenable for 3D spheroid screening approaches or organoid models in cancer research or toxicology.

#### Which surfaces are compatible with the BIOFLOAT<sup>™</sup> FLEX coating solution?

The BIOFLOAT<sup>™</sup> FLEX coating solution is applicable to a broad range of hydrophobic polymer and glass surfaces such as polystyrene (PS), polycarbonate (PC), polydimethylsiloxane (PDMS), polyethersulfone (PES), cycloolefin copolymer, polyvinyliden fluoride (PVDF), polyethylene (PE) and optical quartz glass.

In order to ensure a high affinity of the coating, the culture surfaces MUST NOT be tissue-culture treated. Culture ware compatible with the BIOFLOAT<sup>TM</sup> FLEX coating solution are labeled as non-adherent cell culture or not-treated suspension culture products from common suppliers.

Examples can be found below:

- Sigma Aldrich
- Corning® non-treated culture dishes | Sigma-Aldrich
- Nunc® OmniTray | Sigma-Aldrich
- Greiner: <u>https://shop.gbo.com/en/row/products/bioscience/microplates/96-well-microplates/</u>
- Falcon: <u>https://www.fishersci.com/shop/products/falcon-tissue-culture-plates-4/0877249</u>
- Imaging labware: https://ibidi.com/content/category/19-immunofluorescence-if
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#### How does the BIOFLOAT<sup>™</sup> technology modify the surface of your device?

BIOFLOAT<sup>™</sup> FLEX coating solution instantly creates a monomolecular coating on your surface, which does not modify the surface geometry of your device. Owing to its highly anti-adhesive properties, cell-to-cell interactions are favored, leading to the formation of highly uniform spheroids which float in the medium without interaction with the surface. The coating strongly adheres to the surface and is stable under standard culture conditions including repetitive media exchange.

#### Is the coating with Biofloat<sup>™</sup> Flex quicker than other techniques?

Yes, Biofloat Flex is ready to use and passivates the surface within 3 min and no further steps are necessary (e.g. centrifugation or incubation steps).

#### Does Biofloat<sup>™</sup> Flex passivate only the bottom of the 96-well plate?

Biofloat <sup>TM</sup> passivates the part of the well that it contacts. Using 100  $\mu$ L Biofloat Flex into one well of a 96-well plate coats the well bottom and the wall of the well. The inside of a well of a ready-to use 96 well Biofloat<sup>TM</sup> plate is completely coated – the bottom and the wall.

#### How long is the incubation time of Biofloat<sup>™</sup> Flex?

We recommend 3 min of incubation.

How a Biofloat<sup>™</sup> coating dissolve the issue of aggregates in one well?

Biofloat<sup>™</sup> passivates the complete surface and is highly scratch resistant and mechanically stable. Therefore, the treated surface is completely inert and protein- and cell repellent which impacts the formation of round spheroids without cell aggregates.

#### Are growth factors needed and interact this growth factors with Biofloat<sup>TM</sup>?

The addition of growth factors into the medium is depended on your cell line. If your cells need certain growth factors you can add them into culture. Your protein-based growth factors will not interact with Biofloat<sup>™</sup> because the Biofloat<sup>™</sup> technology is protein- and cell repellent.

#### Does Matrigel<sup>™</sup> interact with the Biofloat<sup>™</sup> coated surface?

No, Matrigel<sup>TM</sup> is a mixture of extracellular proteins and due to the protein-repellent properties of Biofloat<sup>TM</sup> these proteins cannot stick to the surface.

## Why some cell lines need additional proteins such as collagen for spheroid formation?

Adhesion of cells to extracellular matrix proteins via integrins upregulates the expression of cadherins which are responsible for cell-cell contacts.

#### Is it possible to culture organoids in Biofloat<sup>™</sup> plates?

Biofloat was tested for organoid formation from iPSCs but performance depends on your organoid culture. Some organoids need to adhere to extracellular matrix components for maintenance. These organoids can be cultured in Biolfoat<sup>™</sup> plates with addition of extracellular matrices (e.g. Matrigel<sup>™</sup>) but due to the fact that organoids are complicated in culture some of

these organoids have to be embedded in Matrigel<sup>TM</sup>. For this organoids  $Biofloat^{TM}$  is not recommended.

#### Is the BIOFLOAT<sup>™</sup> FLEX coating solution toxic to cells?

BIOFLOAT<sup>™</sup> FLEX coating solution was designed for culture of eukaryotic cells. All products undergo a quality control to ensure the sterility and for the exclusion of bacterial endotoxins. Therefore, our products are not cell toxic and support the formation of vital cell spheroids.

# <u>Are BIOFLOAT<sup>TM</sup> FLEX coated plates compatible for measurements in the</u> plate reader with fluorescent light or UV-light?

BIOFLOAT<sup>™</sup> FLEX coated well-plates are compatible for test systems using fluorescent light (excitation and emission wavelength 380 nm - 780 nm). Furthermore, with BIOFLOAT<sup>™</sup> FLEX also UV-plates can be coated to allow measurements in the range of 260 nm to 280 nm.

#### How long can BIOFLOAT<sup>™</sup> products be stored?

Unopened BIOFLOAT<sup>™</sup> products can be stored up to 18 month. Storage of opened BIOFLOAT<sup>™</sup> products bear the risk of contamination. You can store the opened BIOFLOAT<sup>™</sup> FLEX coating solution flask for four weeks at 4°C. To avoid any contamination, apply the solution as soon as possible in your experiments.

#### Is the BIOFLOAT<sup>™</sup> technology resistant against organic solvents?

Our BIOFLOAT<sup>TM</sup> products were developed for cell culture water-based applications. In waterbased solutions such as cell culture buffers our products are very stable. The BIOFLOAT<sup>TM</sup> FLEX coating solution as well as the coating of the BIOFLOAT<sup>TM</sup> 96-well plate are not resistant against organic solvent e.g. ethanol or DMSO in high concentrations (>1 %(v/v)).

#### Does the coating react with chemicals?

All BIOFLOAT<sup>™</sup> products does not contain any reactive groups, which can react with other chemicals.

#### Is BIOFLOAT<sup>™</sup> a hazardous good?

BIOFLOAT<sup>™</sup> is a nonhazardous good - it contains no substances that are a risk to health. The products are completely biocompatible and non-cell toxic.

#### How is BIOFLOAT<sup>™</sup> disposed properly?

BIOFLOAT<sup>™</sup> disposable cell culture products can be disposed similar as your standard cell culture plates. After using our products in cell culture, the plates or any other used surfaces are autoclaved and discarded in residual waste.

#### Are the BIOFLOAT<sup>TM</sup> products autoclavable?

BIOFLOAT<sup>™</sup> FLEX in the delivered bottle as well as BIOFLOAT<sup>™</sup> 96-well plates are not autoclavable. The BIOFLOAT<sup>™</sup> FLEX coating solution has a boiling temperature of 70°C-100°C. Only BIOFLOAT<sup>™</sup> FLEX treated devices consisting the following materials are autoclavable (121°C, 15 psig for 20 minutes): polymethylpentene (PMP), polypropylene (PP) ,polypropylene Copolymer (PPCO), ethylene-chlorotrifluoroethylene copolymer (ECTFE), fluorinated ethylene propylene (FEP), fluorinated ethylene propylene (FEP), polyfluoroalkoxy (PFA), polytetrafluorethylen (PTFE),polysulfone (PSF), polyetherimide (PEI), polyvinylidenfluoride (PVDF), ethylene propylene rubber (EPR).

### Are the BIOFLOAT<sup>™</sup> products sterilizable?

All products are packaged sterile, further sterilization is not necessary.

Are the BIOFLOAT<sup>™</sup> products chemically resistant? Our precoated 96-well plates consist of polystyrene (PS) and the BIOFLOAT<sup>™</sup> FLEX coating solution is filled in bottles consist of polyethylene terephthalate (PETG). In Tab.2 chemical resistances of PS and PETG are listed.

Tab. 2 List of chemical resistance

Chemicals	PS	PETG
Acids	Yes	No
Basis (inorganic)	Yes	No
Dry salts	Yes	No
Detergens/surfactans	Yes	Yes
Aqueous solutions	Yes	Yes
Media	Yes	Yes