PhenoDrive

What is PhenoDrive?

PhenoDrive are a family of synthetic, peptide-based products specifically designed and produced by Tissue Click Ltd. to promote the appropriate *in-vitro* cell response to meet the needs of your research.

PhenoDrives are an easy to use, one step addition to your current *in-vitro* 2D and 3D culture procedures that, as it is non gel-based, will not interfere with your microscopy or other methods of analysis. PhenoDrives can also be used as an additive for bioinks for 3D cell plotting applications that utilise cells of a neuronal origin.

Application

PhenoDrives are recommended for use as a coating for plastics in traditional 2D culture or the decoration of 3D scaffolds and incorporation into bioinks to promote the expansion, differentiation. And control of cells of mammalian origin.

Storage

PhenoDrives are supplied as a lyophilised powder for easy reconstitution in a range of aqueous or polar solvents. The lyophilised powder can be stored for up to 24 months at -20°C. Reconstituted PhenoDrives can be stored at -20°C and used within 3 months of freezing.

Recommendations for Use

A. Powder Reconstitution Procedure

1. Each vial of PhenoDrive contains 1 mg of freeze-dried product. Dissolve the contents of each vial in 1mL of ethanol or any aqueous medium. Ethanol is recommended for a rapid coating procedure of plastic ware by evaporation

2. Dilute the substrate powder to a concentration of 0.01mg/mL to 0.1mg/mL in any sterile buffer solution pH 7.4 or in 75% ethanol (for rapid coating). The lower concentration (0.01 mg/mL) does not ensure control of cell phenotype beyond 3 days of culturing, while 0.1 mg/mL ensures complete coating of the surface and stability for at least 15 days in tissue culture media

3. Filter the reconstituted solution through a 0.22µm filter

4. Prepare aliquots (e.g. 5 mL) of the 0.1 mg/mL solution in sterile tubes. Each aliquot allows the coating of a 96-well or 24-well plate

5. Use the reconstituted and filtered solution as described in the coating procedure below or store as indicated above

6. NB: All steps must be performed under sterile conditions

B. Cell Culture Well and Scaffold Coating Procedures

Coating of 96 and 24 well plates

1. Use a sterile 96-well or 24-well treated plate under a laminar flow cabinet

2. Pipette reconstituted solution in each well（50 µL for 96 wells, 200 µL for 24 wells)

3. Allow cast coating by solvent evaporation under sterile conditions (UV irradiation is recommended). In the case of use of buffer solution, overnight casting is required. For ethanol

solutions approximately 3 hours are required for casting depending on aeration conditions of the laminar flow cabinet

4. Equilibrate well surface using 2x rapid washes with 100 µL of suitable tissue culture medium without serum supplement

5. Wells are ready for use as per desired experimental protocol. SERUM-FREE SEEDING OF CELLS IS RECOMMENDED. Serum can be added after cell adhesion (e.g. 3 hours after seeding).

Coating of 3D polymer scaffolds

1. Ensure that scaffolds are not soluble in ethanol if substrate solutions are prepared in this solvent

2. Follow steps as described for the powder reconstitution protocol then pipette a sufficient volume to ensure complete coverage of the scaffold. The required volume may change depending on the scaffolds size, porosity, chemical composition and swelling properties. Scaffolds may experience temporary increased swelling when ethanol solutions are used. Equilibration in tissue culture media should restore their original degree of swelling

3. Equilibration in tissue culture media should take into account diffusional constraints related to the scaffolds physicochemical properties. This is particularly important if an ethanol solution is used as it could result in cell cytotoxicity

4. For cell seeding procedure take into account recommendations above

3D Cell Construct Formation in Suspension

1. Solubilise the substrate in the SERUM-FREE tissue culture medium specific for the cell type to be cultured. Reconstitute the powder as described in the powder reconstitution protocol using the same serum-free tissue culture medium to be used for the experiment.

2. Mix the substrate solution with cell suspension to reach a final concentration of substrate ranging from 0.001% to 1% (v/v). A typical mixture with cell suspension varies from 40,000 cells/ml to 1,000,000 cells/ml. Place the sample a non-adherent dish for approximately 20 min at room temperature or 37 ^{o}C on an orbital shaker, transfer the cells to sterile sealed tubes and place them under gentle rotatory conditions for 2h, 37 ^{o}C, in a cell incubator.

3. Seed the cells on plasticware or glassware as normal.

Cell Fixation on Phenodrive Coated Plates

1. Cells may also be fixed with 100% ice cold methanol for 10 minutes or 3.7% formaldehyde solution for 10 minutes at room temperature, however for optimum cell fixation results, 3.7% formaldehyde solution is recommended.

The seeding of the Phenodrive-driven cell constructs in tissue culture plates is recommended to be undertaken after coating the plates with the same PhenoDrive formulation by following the coating procedure described for 2D culturing conditions.

All parameters may need further optimisation by the operator depending on the type of cells and desired experimental conditions.

Note: This product is not intended for clinical use or for implantation in humans or animals.