*Your goal is our goal!*

*Tissue Click Ltd*
*28 Sycamore Close*
*Brighton*
*BN2 6SJ*
*United Kingdom*
*Phone: 0044 (0) 1273302576*
*E-Mail: info@tissueclick.com*
*Web: www.tissueclck.com*

# *TC Specialised Cell Substrates: Instructions for Users*

**Compounds:**

**PhenoDrive - Universal**
**PhenoDrive - Integrin YIGSR**
**PhenoDrive - Integrin IKVAV**
**PhenoDrive- Integrin RGD**
**PhenoDrive - Phosphoserine**
**PhenoDrive - Hypoxia**

**Intended Use:**

- Coating of tissue culture plasticware and glassware
- 3D scaffold surface functionalisation
- cell construct aggregation when used in suspension

Powders have not been tested for combined use.

**Best performances in serum-free seeding and/or culture**.

**Storage Conditions:**
Powder = 4 °C
Reconstituted solution= -20 °C (aliquot storage is advised to avoid repeated freeze/thawing).

## A. Powder Reconstitution Procedure

1. Dissolve substrate powder at a **0.01mg/ml to 1mg/ml** concentration in any sterile buffer solution pH 7.4 or in 75% ethanol (for rapid coating). Low concentration value (0.01 mg/ml) does not ensure control of cell phenotype beyond 3 days of culturing, **while 1 mg/ml ensures complete coating of the surface**.
2. Filter reconstituted solution through 0.22 µm filter
3. Make aliquots (e.g. 5 ml) of the solution in sterile tubes. Each aliquot allows the coating of a 96-well plate
4. Use as described in the coating procedure or store as indicated above
5. All steps to be performed under sterile conditions

**B. Cell culture well coating procedure**

***Coating of 96-well plates***

1. Use a sterile 96-well plate under a laminar flow cabinet
2. Pipette 50 μl of reconstituted solution in each well
3. Allow cast coating by solvent evaporation under sterile conditions (UV irradiation can be applied). In the case of use of buffer solution, overnight casting is required. For ethanol solutions approximately 3 h are required for casting depending on aeration conditions of the laminar flow cabinet.
4. Equilibrate well surface by 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. The specialised substrates also promote cell adhesion in serum-free tissue culture media.

***Coating of 24-well plates***

1. Follow steps as described for the coating of 96-well plates using 150 μl of solution per well

***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 pipetting a volume ensuring complete coverage of the scaffold. The required volume may change depending on the chemical composition of the scaffold, swelling properties, size and its porosity. Scaffolds may experience temporary increased swelling when ethanol solutions are used. Equilibration in tissue culture media should restore their original swelling.
3. Equilibration in tissue culture media should take into account diffusional constraints related to the scaffold physicochemical properties. This is particularly important if an ethanol solution is used as it could result in cell cytotoxicity.
4. **The product intended use is mainly for SERUM-FREE CULTURING CONDITIONS**. IN THOSE CASES WHERE SERUM IS REQUIRED SERUM-FREE SEEDING OF CELLS IS RECOMMENDED. **Serum should be added on after overnight incubation of the scaffold and of the cells in serum free conditions**.

**3D Cell Construct Formation in Suspension (PhenoDrive-Integrin)**

1. Solubilise the substrate in the SERUM-FREE tissue culture medium specific for the cell type to be cultured by reconstituting the vial powder as described in the powder reconstitution protocol.
2. Mix 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 in sterile sealed tubes for approximately 20 min at room temperature or 37 $^{o}$C under gentle rotary conditions.
3. Seed the cells on plastic ware or glassware as normal. **This seeding of the PhenoDrive-driven cell constructs in tissue culture plates is recommended to be done after coating of these surfaces 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.**

**Notes:** The product is not intended for clinical use or for implantation in humans and animals.