# 3D Cell Culture Matrix Kit (Duo-Matrix)

PromoKine

## Instruction Manual

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| **Catalog Number** | PK-CA577-K519 |
| **Description** | Three dimensional (3D) cell cultures are artificially-created environments in which cells are permitted to grow or interact with their surroundings in a 3D fashion. 3D cell cultures improve the function, differentiation and viability of cells and recapitulate in vivo microenvironment compared to conventional 2D cell cultures. 3D matrices provide a physiologically relevant screening platform, by mimicking the in vivo responses, for many cell types including cancer and stem cells in developmental morphogenesis, pharmacology, drug metabolism and drug toxicity studies. PromoKine offers 3D cell culture matrices, including Basement Membrane Matrix (animal-based), Alginate Hydrogel (plant-based) and proprietary PromoKine's Duo-Matrix Mix, to meet the needs and requirements of various research fields. PromoKine's 3D Cell Culture Matrix Kits provide a standardized, yet user friendly and adaptable to high-throughput strategy for setting up spheroid formations, 3D cell cultures and pharmacological studies.<br>**Applications:**<br>• Spheroid formation assays<br>• Adaptable to any 3D cell culture based drug screening studies<br><br>**Sample Type:**<br>• Adherent and suspension cells |
| **Quantity** | 100 assays |
| **Kit Components** | **Components** / **Quantity** / **Cap Code**<br>Duo-Matrix / 5 ml / NM<br>Cross-linking Solution / 25 ml / NM<br>Wash Buffer / 100 ml / NM |
| **User Supplied Reagents and Equipment** | • Cell Culture Media<br>• Clear-bottom, sterile 96-well plates<br>• Microscope<br>• Matrix Dissociation Buffer (Cat No. PK-CA577-M1090)<br><br>For human primary cells and the corresponding optimized cell culture media visit www.promocell.com. For Cytokines/Chemokines and Growth Factors visit www.promokine.info. |
| **Applications / Assay Protocol** | **Storage Conditions and Reagent Preparation:**<br>Store kit at -20°C, protected from light. Read entire protocol before performing the assay. Perform the assay under sterile conditions.<br>• **Duo-Matrix:** Aliquot and store at –20°C. Avoid multiple freeze/thaw. Thaw and keep on ice before use. Use within two months.<br>• **Cross-linking Solution:** Store at -20°C. Thaw and keep at 4°C before use. Stable for six months after the first thaw.<br>• **Wash Buffer:** Store at -20°C or 4°C. Stable for six months after the first thaw. Bring to room temperature (RT) before use.<br><br>**3D Cell Culture Protocol:**<br>**1. Cells:** Grow cells in appropriate media and culture conditions. Adherent cells should be cultured to ~80% confluency. Harvest cells and centrifuge at 1,000 x g, for 5 minutes. Resuspend the cell pellet in Wash Buffer and count the number of cells using a hemocytometer or an automated cell counter. Re-suspend cells in 500 µl of media at the concentration of 2 x $10^6$ cells/ml. For a 96 well-plate, add 500 µl of resuspended cells to 4.5 ml of thawed Duo-Matrix at RT. Mix gently by pipetting, and add 50 µl of cell mixture to each well to get 10,000 cells per well, and incubate for 15 minutes at 37°C.<br>Note: For a scaled-down experiment to 10 wells, add 55 µl of cells in media (2 x $10^6$ cells/ml) to 495 µl of Duo-Matrix. Next, add 50 µl of cell mixture to each well to get 10,000 cells per well.<br>**2. Matrix Preparation:** To solidify the matrix, add 250 µl of Cross-linking Solution to each well. Incubate at RT for 20-30 minutes until matrix is formed. Matrix would appear as a cloudy solid gel (50 µl volume) in the 250 µl clear liquid solution. After the matrix solidification, remove 220 µl of liquid by pipetting, and the Matrix will remain in the bottom of well. Wash three times with 300 µl of Wash Buffer. Add 200-250 µl of appropriate media and allow cells to grow and |

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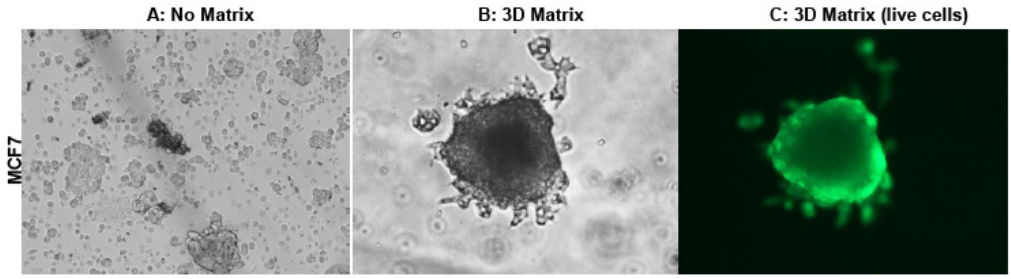
| | form spheroids in 37°C incubator for a set amount of days depending on experimental set up. Change media every 2-3 days.<br>Note:<br>a. Cells typically form spheroids in matrix after 1 week. Matrix remains stable for up to 3 weeks in culture. Vacuum removal of buffer or media could aspirate some or the entire matrix and can cause loss of samples.<br>b. To avoid rupture of matrix, removal of buffer and media by carefully pipetting from the side of the well is strongly recommended.<br>**3. Matrix Dissociation (optional):** Matrix Dissociation Buffer (Cat No. PK-CA577-M1090) is not provided. Add 200-250 µl of Matrix Dissociation Solution. Incubate at RT for 5-10 minutes and then pipet up and down with 1 ml tip until matrix is dissolved. Move the cells and solution to 1.5 ml Eppendorf tubes. To neutralize the Matrix Dissociation Solution, add 1 ml of Wash Buffer to each tube and centrifuge at 1,000 x *g*, for 5 minutes. Resuspend cells in media for use in assay of interest.<br>  <br><br>**Figure:** MCF7 cells in No Matrix (A) and 3D Duo-Matrix (B). Cells were cultured in Duo-Matrix for 21 days, and successfully formed spheroids. Media was changed every 2-3 days as per protocol. The Calcein AM staining (C) indicates that cell viability is not affected while culturing in matrix for a long period of time.<br>Note: Calcein AM (Cat. No. PK-CA707-80011) is not included in the kit. |
| **Storage & Stability** | Store kit at –20°C. |
| **Intended Use** | For in vitro research use only. Not for diagnostic or therapeutic procedures. |

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