3D Cell Culture Scaffold Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K990
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 culture environments improve the function, differentiation and viability of cells and recapitulate in vivo microenvironment compared to conventional 2D cell culture experiments. 3D matrices and scaffolds 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 kits, including Basement Membrane Matrix (BME, animal-based), Alginate Hydrogel (plant-based), proprietary Duo-Matrix and dry Scaffold (natural polymers, animal-free) to meet the needs and requirements of various research fields. PromoKine's ready-to-use 3D Cell Culture Scaffold Complete Kit provides a standardized, and adaptable to high-throughput strategy microplate containing proprietary 3D scaffolds for setting up spheroid formations, 3D cell cultures, pharmacological studies, with an optimized scaffold dissociation method for subsequent biochemical or protein analysis. **Applications:** • Matrix and cell /spheroid dissociations • Cell-harvesting for usage in cell-based assay, biochemical and protein analysis **Sample Type:** • Matrices • Adherent and suspension spheroids and cells
Quantity	100 assays
Kit Components	**Components** — **Quantity** — **Cap Code** Scaffold Dissociation Solution (enzyme-free) — 40 ml — NM Neutralization Buffer — 100 ml — NM Ready-to-Use Scaffolds (96-well plate) — 12 x 8-strips — -
User Supplied Reagents and Equipment	• Cell Culture Media • Microscope • 1.5 ml Eppendorf tubes (sterile) • 96-well trays 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:** Store kit at -20°C, protected from light. Read entire protocol before performing the assay. Perform the assay under sterile conditions. • **Scaffold Dissociation Saline Solution and Neutralization Buffer:** Store at -20°C. Thaw and keep at 4°C before use. Stable for six months after the first thaw. • **Ready-To-Use Scaffolds (96-well plate):** The kit contains 12 detachable 8-well strips and a 96-well tray. Avoid exposure to moisture and store in a dry place at RT. Use within six months. **3D Cell Culture Protocol:** **1. Cell preparation:** 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 Neutralization Buffer and count the number of cells using a hemocytometer or an automated cell counter. Resuspend cells in 500 µl of media at the concentration of 4 x 10^6 cells/ml. For 96 well-plate, add 500 µl of resuspended cells to 4.5 ml of media at RT (4 x 10^5 cells/ml). Note: For a scaled-down experiment to 1 strip of 8 wells, add 50 µl of cells in media (2 x 10^6 cells/ml) to 450 µl of media. **2. Cell Seeding:** Mix gently by pipetting, and gently seed 25 µl of cell mixture (2 x 10^5 cells/ml) to the top of each scaffold to get 10,000 cells per scaffold. Incubate for 0.5-1 hour at 37°C to allow cells to attach to scaffold. Next, gently add 200-250 µl of appropriate media to the side of each well. Allow cells to grow and form spheroids in 37°C incubator for a set amount of days depending on experimental set up. Change media every 2-3 days.

Notes:
a. Cells typically form spheroids in scaffold after 1 week. Scaffold remains stable for up to 3 weeks in culture. Vacuum removal of buffer or media could aspirate some or the entire Scaffold and can cause loss of samples.
b. Careful addition or removal buffer and media (i.e. by tilting the plate at an angle and pipetting from side of well) is strongly recommended to avoid rupture of scaffold or unwanted dissociation of cells from scaffold.

3. Scaffold Dissociation: Once cells/ spheroids are ready to be harvested for subsequent analysis, remove carefully all media, and add 300 µl of Scaffold Dissociation Solution. Incubate at RT for 5-10 minutes and then pipet up and down with 1 ml tip until scaffold is dissolved. Transfer the cells in the solution to 1.5 ml Eppendorf tubes.
Note: If the scaffold doesn't completely dissolve into liquid form in the well, transfer it to clear Eppendorf tubes, and add additional 150 µl of Scaffold Dissociation Solution and incubate for another 5-10 minutes. Vortex at low setting if needed.

4. Neutralization: To neutralize the Scaffold Dissociation Solution, add 700 µl of Neutralization Buffer to each tube, invert tube a few times to mix, and centrifuge at 1,000 x g, for 5 minutes at 4°C. Remove the solution carefully without disrupting the cell pellets. Resuspend cell pellet in Neutralization Buffer or buffer/media of your preference, for future use in assays of interest.

Note:

a. For biochemistry or protein analysis, use ice-cold Dissociation Saline Solution and Neutralization Buffer, and keep samples on ice.

b. For lysate storage, add ice-cold Cell lysis buffer (not provided) to cell pellets, snap freeze samples and store at -80°C.

c. For live-cell storage (freeze down cells), resuspend cell pellets in appropriate cryopreservation solution and store at -80°C.

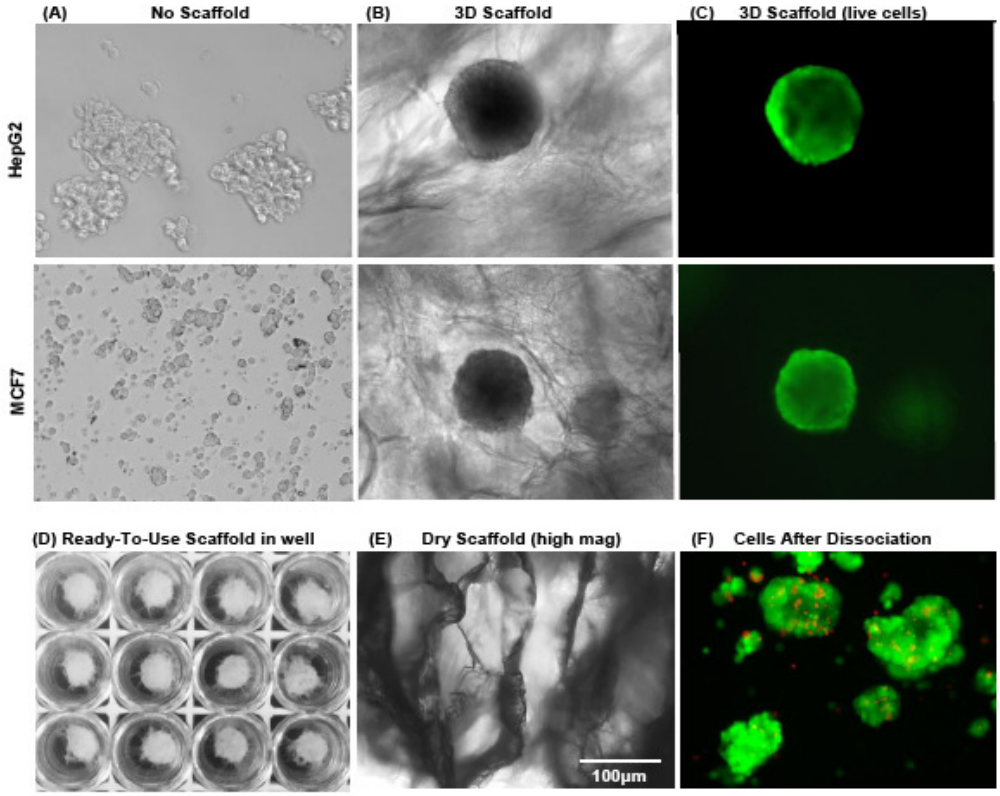

Figure: HepG2 and MCF7 cells in No Scaffold (A) and 3D Scaffold (B). Cells were cultured in Scaffolds for 21 days, and successfully formed spheroids. Media was changed every 2-3 days as per protocol. The Calcein AM staining (C) indicates that cell viability is not affected while culturing in Scaffold for a long period of time. (D) Image of Ready-to-Use dry Scaffold sponges in a 96 well plate. (E) Dry Scaffold without media or cells: high magnification image from light microscopy shows the interconnected pores (50-200 µm in size) within the scaffold. (F) Scaffold dissociation (step 3) was carried out on HEPG2 cells, grown in Scaffold for 21 days. There is no significant cell death (Red: Ethidium Homodimer-1) after dissociation. Viable cells are shown in green (Calcein AM).

	Note: Calcein AM (Cat. No. PK-CA707-80011) and Ethidium Homodimer-1 (PK-CA707-40014) are 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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