Evaluation of Total Collagen

1. Cell Culture Preparation

Expand adult dermal fibroblasts to 80 – 90 % confluence in CnT-PR-F, then passage the cells and seed at a density of 400'000 cells per well (12-well plate) in 1 mL CnT-PR-F. For cell number normalisation using gDNA (step 3), seed additional wells for each treatment/condition.

Change medium approximately 24 h post seeding to 500 uL CnT-PR-ECM per well, and incubate the plate for 5 days without medium change.

2. Collagen Detection

Total soluble collagen is detected with the soluble collagen assay (QuickZyme QZBCOL1) according to the manufacturer's protocol, with some modifications to the sample preparation.

For a more complete evaluation of deposited collagen, it is strongly recommended to evaluate total cell lysates (that include the deposited collagen), as opposed to cell culture supernatants. Begin by removing the cell culture medium, and washing each well with 1 ml PBS. Add 600 uL of 0.5 M acetic acid per well and scrape the cell layer from the base and walls of each well using a pipette tip. Transfer the contents to a 1.5 mL Eppendorf tube.

Perform three snap-freeze cycles in liquid nitrogen with brief vortexing in between. Centrifuge the samples at 3'000g for 10 min 4 °C. Samples are then analysed according to the kit manufacturer's instructions.

If desired, cell culture supernatants can also be analysed in parallel for a secondary insight into collagen production. For these samples, the final centrifugation step in the sample preparation is done at 1'500g.

3. Cell Number Normalization (gDNA)

Remove the culture supernatant from the wells and wash cell with 1 ml PBS. Add 500 ul RLT lysis buffer per well and detach the cell layer from the base and walls of the well using a pipette tip.

Pass the contents of the well, in particular the viscous part, through a 26G needle 15 times in order to shear the genomic DNA. Transfer 10 uL into 96 well plates with at least 3 replicate wells per sample. RLT buffer is used as blank. Add 200 uL of EtBr (0.5 ug/ml in H_2O) per well. Mix well by pipetting and incubate for 5 min. Read the plate at Ex=25 and Em=605.