Protocol for Micromass Culture (Chondrogenic Activity Assay)

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1) Expand cells in T-25 or T-75 flasks (**You may need 0.5 to 5x 10^6 cells per seeding**).
2) Trypsinize cells, add complete medium, and transfer to 15ml or 50ml Falcon tubes.
3) Spin down @ 1000rpm x5 min (use cold room centrifuge, Eppendorf 5810).
4) Aspirate the medium and resuspend cell pellets in 1.0ml complete medium.
5) Transfer to 1.5ml microfuge tubes, and spin @ ~600 to 1000rpm x5 min.
6) Aspirate the medium and resuspend in final volume (= number of wells x volume per well) (**We used 24-well plates and the seeding volume was between 25ul to 50ul per well**).
7) Add cells to center of each well, taking care not to touch the sides of the wells.
8) Carefully transport the plates to CO2 incubators. Allow cells to attach (usually 2 to 4 hours).
9) Add medium carefully to each well (i.e., 1 ml per well for 24-well plates). Do not disturb cells.
10) Proceed with chongenic assays (e.g, Alcian Blue stain etc) at your desired time points.

Notes:

Make sure cells remain in center of well.
For a 24-well plate, 20-50ul is ideal. More than 50ul will spread too much.

Ref:
Sawyer LM. Goetinck PF. Chondrogenesis in the mutant nanomelia. Changes in the fine structure and proteoglycan synthesis in high density limb bud cell cultures. *Journal of Experimental Zoology. 216(1):121-31, 1981 Apr.*

Daniels K. Reiter R. Solursh M. **Micromass** cultures of limb and other mesenchyme. *Methods in Cell Biology. 51:237-47, 1996.*