Example protocol for the culture of the TERA2.cl.SP12 cell line on alvetex® (22 mm disc in 12-well plate format, AMS.AVP002)

Preparation for 3D cell culture on alvetex®

1. TERA2.cl.SP12 cells[1,2] were routinely maintained in T-75 flasks.

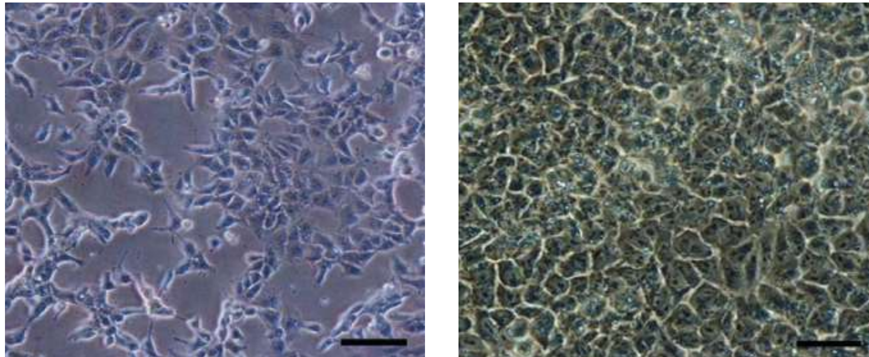
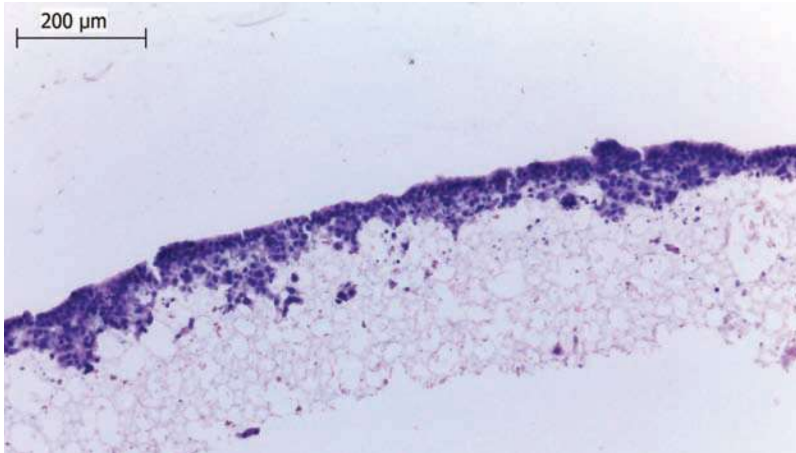


Figure 1. *Phase contrast micrographs of TERA2.cl.SP12 cells grown in conventional 2D culture plates. Images show cells at low (left) and high (right) confluency. Scale bars: 100 µm.*

2. Complete media consisted of: Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% v/v heat-treated FBS, 2 mM L-glutamine and 100 U/ml Penicillin/Streptomycin.
3. Cells were harvested by trypsinisation and centrifuged for 5 minutes (1000 rpm). The supernatant was discarded and the cell pellet was re-suspended in an appropriate volume of media for cell counting by Trypan Blue.
4. Cells were re-suspended at a concentration of 4×10^6 cells/ml for seeding.
5. Alvetex® 12-well plates were prepared for seeding with 70% ethanol (2 ml per well) and media washes (twice with 3 ml of media each) as described in the product information leaflet.
6. 125 µl of the cell suspension was added to the centre of the alvetex® disc, which was equivalent to 500,000 cells per well.
7. The plate was incubated 3 hours at 37 ˚C with 5% CO_2 to allow the cells to settle into the scaffold.
8. 4 ml of media was added to each well taking care not to dislodge cells from alvetex®.
9. Plates were re-incubated and maintained by complete media exchange after every 1-2 days.

[1] Przyborski S.A. (2001). Isolation of human embryonal carcinoma stem cells by immuno-magnetic sorting. Stem Cells, **19**, 500-504.

[2] Stewart R., Christie V. & Przyborski S.A. (2003). Manipulation of human pluripotent embryonal carcinoma stem cells and the development of neural subtypes. Stem Cells, **21**, 248-256.



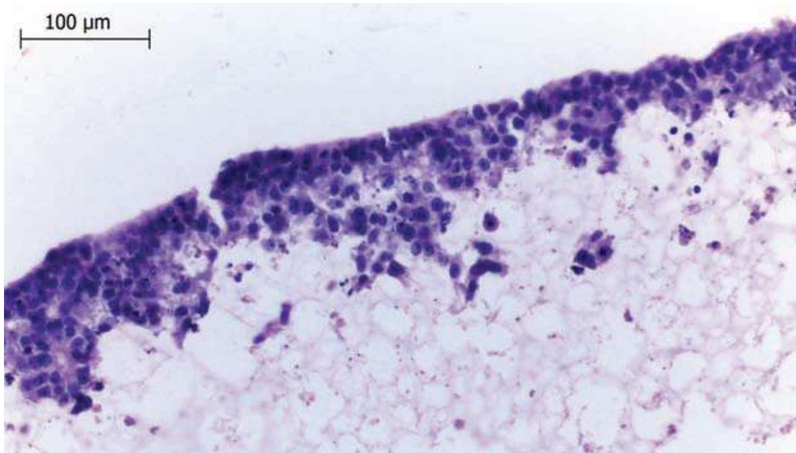


Figure 2. *Brightfield micrographs showing the structure of TERA2.cl.SP12 cells cultured for 7 days on 22 mm diameter alvetex® discs presented in the 12-well plate format. Cells were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin.*

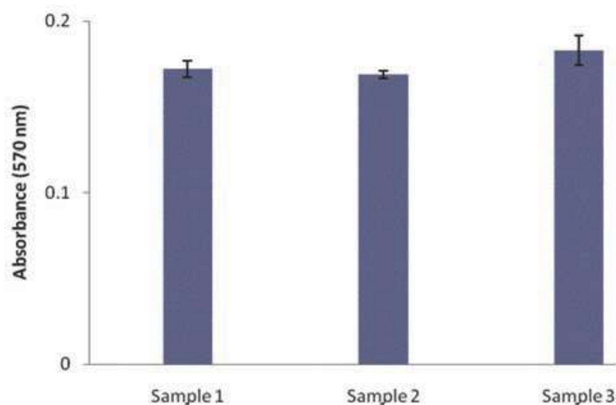


Figure 3. *Biochemical analysis of cell viability using a standard MTT assay. Data from 3 sample replicates of TERA2.cl.SP12 cells are shown (n=3, mean ± SD). Cells were cultured for 3 days on 22 mm alvetex® discs presented in the 12-well plate format.*

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UK & Rest of World
184 Milton Park, Abingdon
OX14 4SE, Oxon, UK
Tel: +44 (0) 1235 828 200
Fax: +44 (0) 1235 820 482

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
Tel: +41 (0) 91 604 55 22
Fax: +41 (0) 91 605 17 85

Deutschland
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
Tel: +49 (0) 69 779099
Fax: +49 (0) 69 13376880

United States
23591 El Toro Rd, Suite #167
Lake Forest, CA 92630
Tel: + 1 800 987 0985
Fax: + 1 949 265 7703

amsbio
info@amsbio.com