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General cell culture guidelines and consumables

For growing AN3-12 ES cells, standard cell culture techniques apply. We use cell culture-treated dishes, mostly from Greiner (15 cm dishes, 15cm Cellstar cell culture dishes, cat no 639160) and nunc (all other formats, e.g. 10cm dish Nunclon Δ Surface, cat no. 150350; 6-well Nunclon Δ Surface, cat no. 140675), but any other supplier will most likely also be fine. Coating of dishes, i.e. w/ gelatin, is not required.

ES cell medium (ESCM)

450 ml DMEM Sigma D1152
75 ml FCS Invitrogen
5.5 ml P/S Sigma P0781
5.5 ml NEAA Sigma M7145
5.5 ml LGlu Sigma G7513
5.5 ml NaPyr Sigma S8636
0.55 ml bME Merck 805740 (dilute 10 ul bME in 2.85 ml PBS for a 1000x stock)
ESGRO Millipore ESG1107 (acc. to instructions by manufacturer)

We usually do a FCS test assuring that we select a batch which allows for rapid proliferation and a high rate of colony formation without massively inducing differentiation of our ES cell lines. We usually test around 3-5 different batches, depending on availability.

1x Freezing medium (FM 10% DMSO)

5 ml ESCM
4 ml FCS
1 ml DMSO Sigma 41648

2x Freezing medium (20% DMSO)

4 ml FCS
1 ml DMSO

We feed AN3-12 cells every day with fresh ESCM and split them before they will get too dense and confluent, respectively. As a rule of thumb, the split ratios are about 1:3 if you need the cells to be ready the next day (i.e. Mon to Tue), and a ratio of 1:10 if you need them another day later (i.e. Mon to Wed). A ratio of 1:30-1:40 is sufficient to get them over the weekend and feeding them once on Sunday.

For reviving cells, thaw cells as quickly as possible, e.g. in a 37°C water bath, transfer the cell suspension to a Falcon tube already containing ESCM, pellet the cells (310g, 5min),

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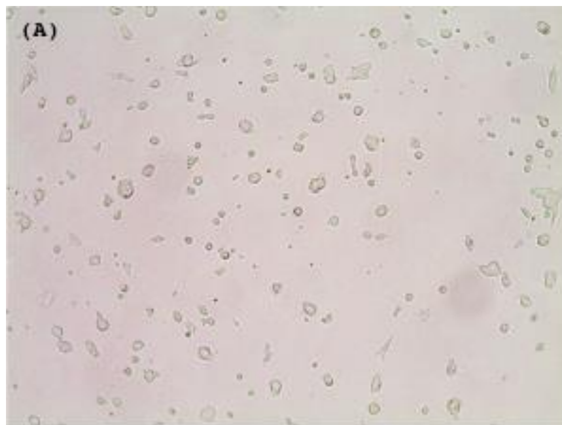
carefully resuspend the cell pellet in ESCM and plate the cells accordingly, i.e. on 1x6w if frozen down in a 2D Matrix tube, and 1x10cm if frozen down in 2ml cryo vials.

A typical visual representation of wildtype AN3-12 cells is shown after (A) 20h, and (B) 70h post thawing. Should you experience any problems reviving wildtype cells and clones, respectively, please feel free to contact us by email.



To trypsinize cells, wash once w/ PBS, add enough 1x Trypsin (e.g. Invitrogen 10x stock, 15400-054) to the cells to cover them, and incubate for about 6 min at 37°C before you inhibit trypsin by adding ESCM in excess. Singularize the cells by pipetting cells reasonably vigorously up and down for a few times, before pelleting the cells (310g, 5min), resuspending them in ESCM, and seeding the cells in fresh ESCM according to your needs.

For freezing cells, trypsinize, stop, pellet the cells, and resuspend cells either in ESCM and subsequently add an equal volume of 2x FM, or alternatively, resuspend the cells directly in 1x FM. Cells are aliquoted into cryogenic vials (e.g. CryoTube Vials, Thermo Scientific), transferred to a freezing container (e.g. Nalgene Cryo1°C Freezing Container, Cat. No. 5100-0001), which is kept at -80°C o/n, before cryo vials are transferred to liquid nitrogen for long term storage.

For colonies to form from single cells, it takes about 10 days, i.e. colonies are nicely visible by eye and have the optimal size to pick (e.g. to generate clones or to generate subclones, etc.).

For picking colonies manually, wash the cells once w/ PBS, and pick colonies in 20µl PBS under a microscope in a 96U well by sucking up and/or slightly scraping the colonies off of the dish. To make single cells from picked colonies, add 5µl of 5x trypsin to the colony in 20µl of PBS, incubate the cells for about 6 min at 37deg, stop w/ 175µl of ESCM and split the colonies according to your needs, e.g. 1:5 in a 96F well, or directly into a 24w

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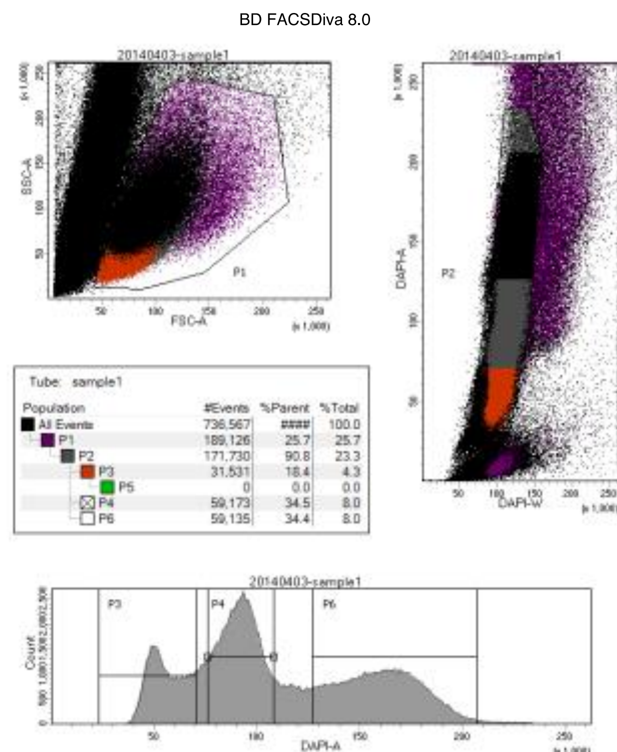
Please keep in mind that the wildtype AN3-12 cells we provide become diploid over time, the rate for which was estimated to be ~2-3% for each day in culture (Elling et al. (2011), Cell Stem Cell, 9(6): 563-74). Consequently, should your intended experiments require a large number of "pure" haploid ES cells, you need to FACS sort the cells by gating for the "1n haploid" peak.

To prepare cells growing on a 15cm dish for FACS sorting, wash the cells w/ PBS, trypsinize the cells w/ 6 ml of 1x trypsin for 5-6min at 37°C, stop the reaction w/ 12ml of ESCM, carefully and very thoroughly make a single cell suspension and add 30µl of Hoechst DNA stain (10mg/ml Hoechst 33342 stock, Sigma B2261) directly to the cell suspension and immediately swirling the plate to distribute the dye. Subsequently, add another 12ml of ESCM (total vol 30ml), mix the cells again on the plate, and incubate for 30-40min at 37° C. To avoid settling of the cells during staining, occasionally mix the cells by gently swirling the plate. After staining, collect and pellet the cells, resuspend them in ~500µl of ESCM, pass them through the cell-strainer cap of a FACS tube (Falcon 352235), and keep them on ice until FACS sorting. Shortly after thawing, i.e. after 1-2d, the wildtype AN3-12 cells we provide should give you a FACS profile as follows:



Collect the haploid peak (P3) in ESCM (e.g. 8ml of ESCM in a 15ml collection tube), and pellet the cells prior to plating. It is advisable to exchange the ESCM after around 8h in order to increase the viability after FACS sorting.

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Again, should you experience any problems FACS sorting wildtype cells, please feel free to contact us by email.

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