Recommendations for the cultivation of CADO-ES1 cells

Growth medium

DMEM:Ham's F12 (1:1, vol:vol), supplemented L-glutamine, 15 mM Hepes, 1 mM sodium pyruvate and 5% FBS. (MG-40, CLS Cat.-no. 820401, ready-to-use)

Rinsing solution: DPBS, without calcium / magnesium (e.g. PBS, CLS Cat.-no. 860015)

Passaging solution: Accutase (CLS Cat.-no. 830100, 100 ml)

CADO-ES1

One cryovial, deep-frozen, order no. 300127;
Current Lot-no. 300127-416SF, Passage: 37, 2 Mio. cells/ml, 1.5 ml.

Disposables

Cell culture flasks (T25= 25cm^2; T75=75cm^2 culture area), sterile serological pipettes.

Thawing of CADO-ES1

Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent.

As soon as the sample has thawed, remove the cryovial from the water bath. Note: A small ice clump should still remain and the vial should still be cold.

From now on, all operations should be carried out under aseptic conditions.

Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol.

Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of growth medium at room temperature.

Resuspend the cells carefully.

Centrifuge at 300xg for 3 min and discard the supernatant.

Resuspend the cells carefully in 10ml fresh cell culture medium and transfer the cell suspension into two T25 cell culture flasks, 5 ml each.

The final cell density at this stage is about 1 x 10^5 cells/cm^2. The viability measured after thawing is >90% viable cells.

First subculture step

About 48 hrs after thawing, the cell sheet is almost 100% confluent.

Remove the spent medium and rinse the adherent cells using PBS without calcium and magnesium. Use 3-5 ml PBS for each T25 cell culture flask.

Add 1 ml Accutase per T25, the cell sheet must be covered completely.

Incubate at ambient temperature for 8-10 minutes.

Carefully resuspend the cells using 4 ml of growth medium to end up with a final volume of 5 ml.

Count the cells.

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Dispense the cells at a cell density of 1 x 10^4/cm^2 in T75 cell culture flasks. You should have roughly 6x T75 cell culture flasks at this stage.

Subculture routine of CADO-ES1

The spent media should be replaced with fresh media every 2-3 days.

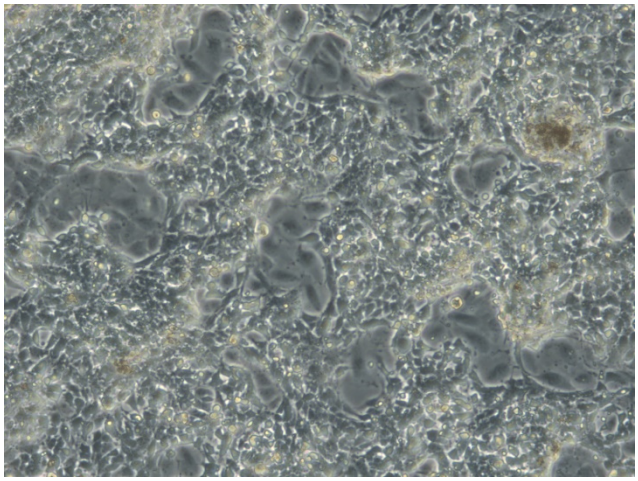
Once the cell sheet is about 90% confluent, repeat the steps using Accutase as described above. Do not subculture at an earlier stage.

Rinse the cell sheet using 5 ml PBS for each T75 cell culture flask and 10 ml PBS for each T150 cell culture flask.

Add 2.5 ml Accutase per T75, 5 ml per T150.

Always incubate at ambient temperature for 8-10 minutes.

Dispense the cells at a cell density of 1 x 10^4/cm^2 into T75 or T150 cell culture flasks or tissue culture flasks of other sizes.



The CADO-ES1 cell line is a heterogeneous mixture representing three morphologies. Ewing's sarcoma cells are described as being of small round shape. Be careful not to dilute the cell line below the recommended cell density, as this may give rise to fibroblasts only.

Freezing of CADO-ES1

Once the cell sheet is about 80% confluent, detach the cells as described above.

Collect the cells in growth media and count.

Centrifuge at 300xg for 3 min and discard the supernatant.

Resuspend the cells in the appropriate volume of freeze medium (CM-1, Cat.-no. 800125, 25 ml, 800150, 50 ml; CM-ACF, serum-free, 800625, 25 ml, 800650, 50 ml).

A cell density of 2 Mio./ml is recommended for this protocol. Higher cell densities are tolerable.

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