GENOMICS

HAP1 Cell Culture Guidelines

HAP1 cells are cultured in Iscove's Modified Dulbecco's Medium (IMDM) with 10% FCS and 1% Pen/Strep. Always be gentle when resuspending cells to avoid any mechanical stress. Each knockout cell line may grow at different doubling times and cells may have a slightly different morphology.

Recommended Products

Iscove's Modified Dulbecco's Medium (IMDM):
GIBCO, Cat.No. 12440-053 (500ml)
Fetal Bovine Serum: Sigma Aldrich, Cat.No. F2442-500ML
Trypsin-EDTA Solution (1X): GIBCO, Cat.No. 25300096 (100 ml)
Penicillin / Streptomycin: GIBCO, Cat.No. 15140-122 (100 ml)

Media for Freezing

Medium A: IMDM + 20 % FBS
Medium B: IMDM + 20 % FBS + 20 % DMSO

Thawing of HAP1 Cells

1. Thaw the vial with frozen cells quickly. You may do this by placing the vial in the 37 °C waterbath.
2. Dilute cells in 10 mL of pre-warmed culture medium.
3. Optional: Spin down cells for 5 minutes at 1200rpm/300 x g. Aspirate medium without disturbing the pellet and add 10 mL fresh medium.
4. Transfer cells to a 10 cm dish.
5. Monitor cells closely for the next 2 days.
6. Change medium after 24 hours.
7. Please see the pictures below for examples of how cells will look 48 hours after thawing.

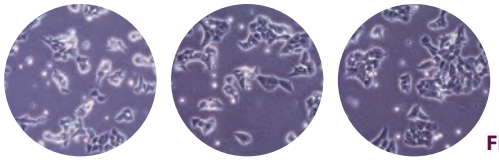

Fig.1 HAP1 clones 48 hours post-thawing

Passage / Culture of HAP1 Cells

1. Aspirate medium from cells.
2. Wash with PBS to remove all traces of medium and FBS.
3. Add Trypsin (0.05 %) and incubate at 37 °C until cells begin to detach (usually 3 to 5 minutes).
4. Add medium to stop trypsinization. Resuspend cells and transfer desired amount of cells to a new dish.

Note: HAP1 cells should be split 1:10 to 1:15 every 2 to 3 days. Keep in mind that growth rates may vary between different clonal HAP1 cell lines. HAP1 cells should never be kept at a high density (maximum density: 75 % confluency). See pictures below for examples of low density and high density HAP1.

Freezing HAP1 Cells

1. Trypsinize and spin down cells for 5 minutes at 1200rpm/300 x g.
2. For freezing cells, use a 1:1 mixture of Medium A: Medium B. First, resuspend cell pellet in Medium A.
3. Slowly add Medium B.
4. Transfer cell suspension to suitable cryovials.
5. Be sure to transfer the cryovials to the freezer within five minutes after addition of DMSO-containing medium. Place tubes in a suitable freezing container in a – 80 °C freezer in order to allow slow cooling.
6. The following day, transfer cells to liquid nitrogen.

Note: Using Medium A and B allows more flexibility when preparing many samples for freezing. If few samples are being prepared, use a complete freezing medium consisting of IMDM + 20 % FBS + 10 % DMSO. After step 1, resuspend cells 1 mL of complete freezing medium and continue to step 4.

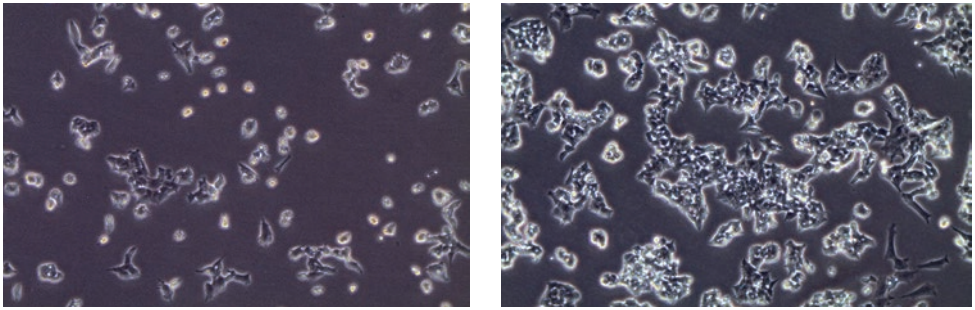

Fig.2 HAP1 cells at low density (left panel) and high density (right panel)

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