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.

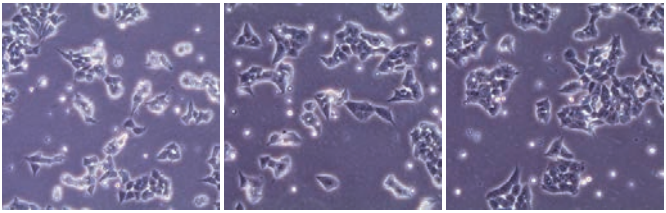

Fig.1
HAP1 clones 48 hours post-thawing

t +43 1 9165522 140
f +43 1 9165522 16
e genomics@horizondiscovery.com
w www.horizon-genomics.com
Horizon Genomics GmbH, Campus Vienna Biocenter 3, 1030 Vienna, Austria

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.

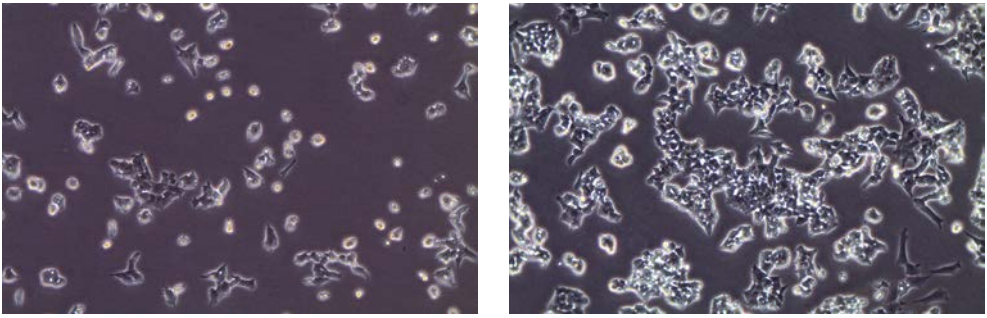

Fig.2. HAP1 cells at low density (left panel) and high density (right panel)

t + 44 (0)1223 655 580 (UK) or +1 (844) 655-7800 (USA)
f + 44 (0)1223 655581
e info@horizondiscovery.com
w www.horizondiscovery.com
Horizon Discovery, 7100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

HAP1 cell culture protocol (TIER 2) - supplement to Horizon SOP

HAP1 haploid human cells for 4DN (from Bas Van Steensel, August 12, 2016)

A. Obtaining the cells.

Each lab obtains the cells from the Netherlands Cancer Institute (NKI) after signing an MTA. In this scenario, the van Steensel lab will take care of the initial expansion, freezing and quality control, according to protocols and advice from Thijn Brummelkamp (also NKI), who invented/generated the HAP1 line and whose lab is using it on a daily basis.

B. Using the cells.

Cells are cultured in IMDM medium supplemented with 10% FCS. The cells do not need to grow confluent and require fresh medium every 2 days. Because the cells spontaneously become diploid, there are procedures to (1) check for ploidy and (2) obtain a near-homogeneous pool of haploid cells:

1. Routine checking by simple FACS analysis of ploidy.

This must be done every week of continuous passaging. 4DN needs to decide on a cutoff, but Thijn and I suggest that <5% diploid cells is acceptable and realistic. All 4DN datasets generated should be accompanied by documentation of the % diploid cells in the samples.

>FACS analysis: typically take ±500,000 cells; spin them down and resuspend in 500 µl Nicoletti buffer. Analyze 10,000 cells by FACS. Diploid HCT116 cells are taken along as reference standard.

Nicoletti buffer 500 ml (in water):

-25 mg Propidium Iodide

-500 ul Triton-X100

-500 mg sodium citrate

2. Selection/expansion of haploid cells.

- limiting dilution of cells in a 96-well plate
- expand clones to 10cm dish
- check ~50 clones for ploidy by FACS analysis according to (1)
- pool >5 clones that have <1% diploid cells
- expand the pool up to 100-500 million
- check the expanded pool for diploid content (<5%) according to (1)
- freeze vials of 20M cells, or use the cells right away

This entire procedure takes about 4 weeks.

Note that these protocols require FACS *analysis*, but not FACS *sorting* of cells.

ADDITIONAL INFORMATION FROM CHRISTINE:

Based on protocol from Horizon Genomics:
https://www.horizondiscovery.com/resources/scientific-literature/faq/how-do-i-culture-my-hap1-cells

Recommended Products
Iscove's Modified Dulbecco's Medium (IMDM): GIBCO, Cat.No. 12440053 (500 ml)
Fetal Bovine Serum: VWR, Cat.No. 89510-186 (500 ml)
Penicillin/Streptomycin: Invitrogen, Cat.No. 15140122 (100 ml)
Trypsin-EDTA Solution (1X, 0.05%): GIBCO, Cat.No. 25300054 (100 ml)
Dulbecco's Phosphate Buffered Saline (DPBS): GIBCO, Cat.No. 14190144 (500 ml)

Medium for Culturing
IMDM + 10% FBS + 1x Penicillin/Streptomycin

Media for Freezing
Medium A: IMDM + 20% FBS
Medium B: IMDM + 20% FBS + 20% DMSO

Note: Filter sterilize all media before use.

Thawing HAP1 Cells
1. Thaw the vial with frozen cells as quickly as possible. You may do this by placing the vial in the 37 °C water bath.
2. Immediately dilute cells in 10 mL of pre-warmed culture medium.
3. Spin down cells for 5 min 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 h.

Please see the pictures below for examples of how cells will look 48 h after thawing.

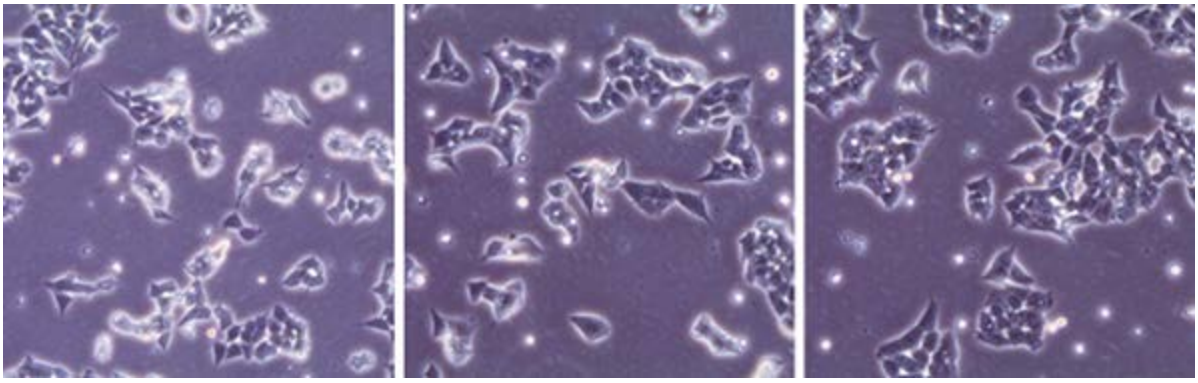

Figure 1. HAP1 clones 48 h post-thawing

Passage / Culture of HAP1 Cells

1. Aspirate medium from cells.
2. Wash with DPBS to remove all traces of medium and FBS.
3. Add trypsin (0.05%) and incubate at 37°C until cells begin to detach (about 3 min).
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 every 2 days or if necessary 1:20 every 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.

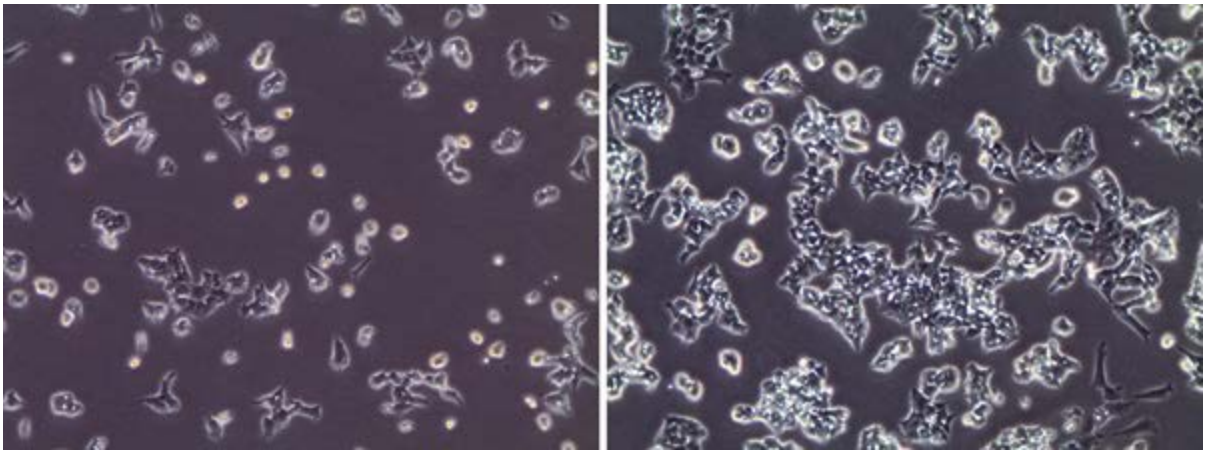

Figure 2. HAP1 cells at low density (left panel) and high density (right panel)

Freezing HAP1 Cells

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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 in 1 mL of complete freezing medium and continue to step 4.