PROTOCOL

HAP1 Cell Culture Guidelines

Production

The Horizon HAP1 cell lines are generated using CRISPR/Cas9 editing. All HAP1 cell lines are supplied as a single vial. See the cell line specific Certificate of Analysis (CoA) for exact freezing details. Each HAP1 cell line is also supplied with the parental cell line as an additional control. Horizon HAP1 cell lines are Biological Safety Level 1 (BSL-1).

Shipping and storage

Horizon HAP1 cell lines ship on dry ice. While precautions have been taken to prevent CO_2 from entering the vial during shipment, it is suggested that upon receipt the cells be stored for two days or more in liquid nitrogen to allow any CO_2 to dissipate. When removing the vial from liquid nitrogen storage, leave at room temperature for approximately 30 seconds or longer to allow the liquid nitrogen to dissipate from the vial. **Note: Always wear protective eyewear and gloves when handling vials stored in liquid nitrogen.**

Starting cells from frozen cell stock (thaw quickly)

The following instructions are for resuspension in 10 cm plate. A similar protocol with different resuspension volumes could be used to start cells in another appropriately sized vessel (i.e. T25/T75 flask).

1. Remove the HAP1 cell line from liquid nitrogen and place in a 37°C water bath for 2 minutes until nearly (~80%) thawed.
2. Dilute cells in 10 mL of pre-warmed culture medium.
3. Optional: Spin down cells for 5 minutes at 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.

Note: Photos provided are of wild-type HAP1 cells. Please note knock-out cell lines may have different phenotypes including morphology and attachment. Observe cells upon thawing and culture cells with respect to their growth phenotype, understanding they may grow quicker/slower or more/less adherent than wildtype.

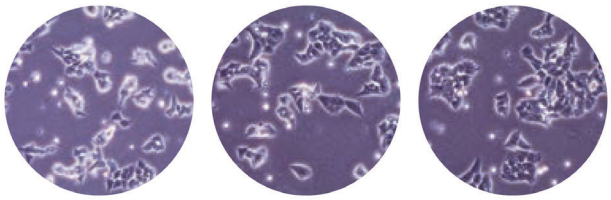

Figure 1. Examples of HAP1 clones 48 hours post-thawing

Sub-culturing cells

We recommend that cells are passaged at least once before using them in your desired application. Growth rates may vary between different knockout clonal HAP1 cell lines and parental cell lines. HAP1 cells should never be kept at high density (maximum density: 75% confluency). See Fig. 2 for examples of low and high density HAP1 cells.

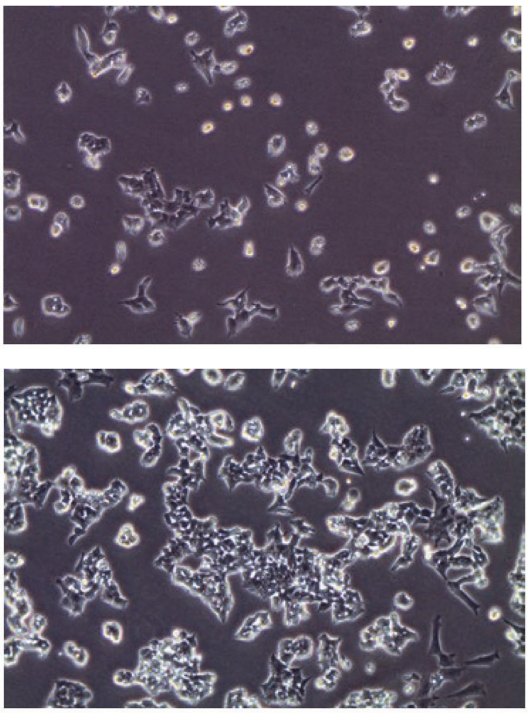

Figure 2. HAP1 cells at low density (top panel) and high density (bottom panel)

HAP1 Culture Medium and Maintenance

HAP1 cells are cultured in IMDM with 10% FBS and 1% Pen/Strep. Always be gentle when re-suspending cells to avoid any mechanical stress. Each knockout cell line may grow at different doubling times and cells may have a slightly different morphology. HAP1 cells should be split 1:10 to 1:15 every 2 to 3 days based on growth.

1. Carefully aspirate the growth medium from the cells. This is best done by tilting the flask or plate and removing the medium without touching the cell surface. **Note: If cells are not fully attached, half-media changes may be appropriate.**
2. Gently 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.

Technical Tips: We strongly recommend freezing down and using cells at lower passage numbers.

Freezing HAP1 cells

Media for freezing

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

1. Trypsinize and spin down cells at 300 x g for 3 minutes.
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. 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.

Abbreviation and catalog numbers:
IMEM: Iscove'sIscove's Modified Dulbecco's Medium (GIBCO, Cat.No. 12440-053)
FBS: Fetal Bovine Serum (Sigma Aldrich, Cat.No. F2442)
Trypsin: Trypsin-EDTA Solution (GIBCO, Cat.No. 25300096)
Pen/Strep: Penicillin / Streptomycin (GIBCO, Cat.No. 15140-122)

Frequently Asked Questions (FAQ)

1. What happens if I exceed the cell densities recommended?

Exceeding maximum recommended density will affect health and viability of the cells. If cell densities are exceeded, the best practice is to start with a fresh stock.

2. Have Horizon HAP1 cells been screened for HIV1/2, HBV, and HCV?

Both KBM-7 and HAP1 have been classified as BSL1 agents based on publically available information. In addition, Horizon HAP1, KBM7 and EHAP parental cell line stocks were screened for the presence of HIV-1, HIV-2, HBV and HCV. None of these viruses were detectable in these cell lines.

3.**Are mutant cell lines provided with a wild type control?**

Cell lines are provided as isogenic pairs - the modified mutant line and the originating wild type parental line (C631). Additional cell lines in the HAP-1 lineage include a screening ready HAP1 (C859), eHAP (C669) and KBM-7 (C628) are available to purchase separately..

4. How was my knockout confirmed? Will my cells express a gRNA or Cas9?

HAP1 knockouts were produced with transient introduction of CRISPR/Cas9 components, clonally grown and sequenced. The clone should lose expression of the vectors introduced to perform the gene editing. Sequencing was used to confirm that a frameshift or premature stop codon was introduced. Additional characterization and validation of antibodies may need to be done to characterize a phenotype compared to wild-type.

5. Will HAP1 cells diploidize?

HAP1 cells will spontaneously diploidize over time. The knockout will not be affected by diploidization as it is a duplication of the edited allele that gives rise to the diploid state. The haploid line is used to ease production of an edited cell line, maintaining a haploid state is not necessary for most use cases.

If a more haploid cell line is desired, maintaining cell stocks at a low passage number and sorting for the population of smaller cells may help reduce the number of diploid cells in the population. Additional information can be found in this article (Beigl et al. Efficient and crucial quality control of HAP1 cell ploidy status. Biology Open. 2020)

References

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4. Essletzbichler, P. *et al.* Megabase-scale deletion using CRISPR/Cas9 to generate a fully haploid human cell line. *Genome Res* **24**, 2059–2065 (2014).
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7. Lackner, D. H. *et al.* A generic strategy for CRISPR-Cas9-mediated gene tagging. *Nat Commun* **6**, 10237 (2015).

For more information

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