Q&A ATCC® Excellence in Research Webinar "*Discovering ATCC hematopoietic progenitor cells – model systems to study the immune and cardiovascular systems*"

1. Will we be able to download the presentation?
   This presentation will be available to watch on demand on the ATCC website, or click here.

2. Are your primary cells pooled from multiple donors?
   Most ATCC primary cells are from single donors. However, we do have pooled normal Human Primary Umbilical Vein Endothelial Cells (ATCC® No. PCS-100-013), which are derived from 10 individual donors, minimizing the lot-to-lot variability associated with cells derived from single donors.

3. What basal media do you recommend for culturing peripheral blood mononuclear cells (PBMCs)?
   The basal medium for PBMC culture is application-specific; however, RPMI 1640 (ATCC® No. 30-2001) supplemented with 5-10% fetal bovine serum (ATCC® No. 30-2020) is commonly used.

4. Do you offer CD133+ hematopoietic stem cells?
   CD133+ is another surface marker for stem-like and progenitor cells, particularly in the area of cancer stem cells. We do not currently offer cryopreserved CD133+ cells and have not tested its expression.

5. Are the cells isolated from mobilized blood?
   ATCC does not use cells from donors injected with granulocyte colony stimulating factor.

6. Do you have plans to offer peripheral blood derived CD34+?
   We do not currently have plans to offer peripheral blood derived CD34+ cells. We do have the normal Human Primary Bone Marrow CD34+ Cells (ATCC® No. PCS-800-012) and normal Human Primary Cord Blood CD34+ Cells (ATCC® No. PCS-800-014) available.

7. Is negative immunomagnetic selection available for CD34+ CD14+ cells?
   Negative immunomagnetic selection for these subsets of cells is not available at this time. Please contact ATCC Technical Support at tech@atcc.org so that we may be able to assist you further with your specific requirements.

8. Can lots from donors with specific characteristics be requested? *e.g.*, based on sex, age, blood type, or ethnicity?
   Please contact ATCC Technical support at tech@atcc.org so that we may be able to assist you further for requests for primary cells from donors with specific characteristics.

9. Do you have data on marker expression in PBMCs before and after cryopreservation?

We have not tested changes in marker expression in freshly isolated PBMCs or after cryopreservation. There is published literature suggesting that cryopreservation can modulate the expression of some cell surface markers and reduce apparent cell counts, particularly CD62L+ and CD4+ CD25+ FoxP3+ cells; although, some of the results are contradictory, most likely due to differences in cryopreservation methodologies. We recommend that if these markers are critical for your application to use freshly isolated PBMCs.

10. Do you recommend resting cryopreserved PBMCs before use?
    The literature is conflicting on the impact of a rest period on PBMCs, particularly in the context of T-cell function and immunophenotype. Published protocols vary from no rest, to a rest of several hours, to overnight. There are reports that a rest period may alter T-cell phenotype. Therefore, it is up to the end user to determine whether a rest period will or will not impact their specific application with PBMCs.

11. What is the doubling time of CD34+ cells in culture?
    The proliferation of CD34+ cells in culture may vary greatly based on the growth media that the cells are maintained in. The choice of growth media used for maintenance of the cells should be designed to selectively promote the expansion of CD34+ cells without inducing differentiation. In the context of cytokine-induced differentiation, we have observed expansion of the total cell count ranging from 25-fold to over a thousand-fold within a week.

12. Do you have additional marker data on CD34+ cells?
    We have not assessed CD34+ cells for markers other than CD34 and CD45. The makeup of specific CD34+ subtypes contained within each vial of cells will vary due to donor and lot variability.

13. Do you have data on the monocyte subsets present in the CD14+ monocytes?
    We have only assessed the normal Human Primary Peripheral Blood CD14+ Monocytes (ATCC® No. PCS-800-010) for expression of CD45.

14. What is the purpose of swirling and washing the plate repeatedly in the protocol for the generation of macrophages from primary CD14+ monocytes?
    We recommend swirling the plate vigorously to remove loosely attached cells and potentially contaminating lymphocytes and other unwanted cell types (that are not adherent). This step is critical only when using cells that are not enriched for monocytes, such as PBMCs.

15. How do you harvest highly adherent macrophages?
    We incubate the cells in EDTA in cold PBS on ice for 15-20 minutes. Pipetting gently up and down, or using a cell scraper, will release the macrophages. We do not recommend the use of trypsin, which can damage the cells. If you are still experiencing difficulties harvesting the cells you may also try low-attachment plates.

16. Where did you source your cytokine cocktails?
    STEMCELL Technologies. More information may be found in the FAQ section on the ATCC website for the expansion/differentiation of CD34+ primary cells.

17. What viruses are included in the screening?
    Primary immune cells are screened for the following human pathogenic viruses: Hepatitis B, Hepatitis C, HIV (I/II) and HTLV(I/II). More information on ATCC's virus testing may be found on the Certificate of Analysis for each lot of cells.

18. CD34 is also a marker for induced pluripotent stem cells and embryonic stem cells. Did you compare your CD34 positive cells with either of these stem cell types?
    No, we have not performed any side-by-side comparisons of CD34+ hematopoietic cells with other types of stem cells.

19. How does the CD11b expression (among other surface markers you checked) of the pan-myeloid differentiation cells compare to primary cells (freshly isolated from bone marrow or peripheral blood)?
    We have not assessed CD11b expression in the mononuclear cells. Frequencies of specific cell types, as assessed by surface marker expression, will vary from donor to donor. Expressions of other surface markers (*e.g.* CD3, CD8) tested are reported on the Certificate of Analysis.

20. Are you monitoring proliferation of CD34+ cells within the population of differentiating cells? (Do the numbers of cells change while the cells are cultured in the differentiation media?
    Expansion, as measured by total nucleated cells (TNC), was monitored during differentiation. Depending on the cytokine cocktail used, we observed expansion of TNC ranging from 25-fold to over 1000-fold.

21. What is the most abundant CD34+ cells source, cord blood or bone marrow cells? Are there differences in the source of CD34+ cells?
    The highest yield of total CD34+ cells from a single donor typically comes from adult bone marrow tissue. Phenotypic differences between CD34+ cord blood-derived versus bone marrow-derived cells have been reported. Refer to the following literature for more information:
    1. Kim D, *et al.* Comparison of hematopoietic activities of human bone marrow and umbilical cord blood CD34 positive and negative cells. *Stem Cells* 17(5):286-294, 1999.
    2. Kita K, *et al.* Cord blood-derived hematopoietic stem/progenitor cells: current challenges in engraftment, infection, and *ex vivo* expansion. *Stem Cells Int*, 2011.
    3. Yang S, *et al.* Hematopoietic reconstitution of CD34+ cells derived from short-term cultured cord blood mononuclear cells. *Biotech Bioprocess Eng* 14(4):429-435, 2009.

22. Do you have information on lead times for these products? What is typical?
    We currently have the primary cell products in stock and they are available to ship immediately.

23. Do you have CD34+ cells selected from patients with histories of cancer?
    No, at this time ATCC does not offer CD34+ cells from diseased state donors.

24. Do you do mycoplasma testing on your cells?
    ATCC primary hematopoietic cells are isolated directly from screened, healthy human donors according to strict AABB and FDA guidelines and immediately cryopreserved so the likelihood of mycoplasma infection is sufficiently low, therefore our primary hematopoietic cells are not tested for mycoplasma. If you wish to request a mycoplasma tested lot, or to request testing of a specific lot please contact ATCC Technical Support (tech@atcc.org). If you wish to test for the presence of mycoplasma in your cells we offer a Universal Mycoplasma Detection Kit (ATCC® No. 30-1012K) for purchase. We also offer ATCC's Mycoplasma Testing Service (www.atcc.org/Services/Testing_Services/Mycoplasma_Testing.aspx) where you may send us your cell culture samples for mycoplasma testing.

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