



Q&A ATCC® Excellence in Research Webinar “Stem Cell Solutions”

General Questions

1. Will we be able to download the presentation?

This presentation will be available to watch on demand [here](#).

2. Are these cell lines referenced in the literature?

Many of the mouse ES cell lines are referenced in the literature. The human Mesenchymal stem cells (MSCs) and human induced pluripotent stem cell (iPSC) lines were recently developed at ATCC. A search of recent research publications did not turn up any references for either the MSC or the iPSC lines.

3. Do you use STR genotyping characterization to authenticate your cells in addition to karyotyping?

Yes, we do use STR analysis along with karyotyping to authenticate the human iPSC lines. The results of the STR analysis are included on the lot specific certificate of analysis for each human iPSC culture.

Induced Pluripotent Stem Cell Questions

4. Is it necessary to keep iPSCs that have been reprogrammed using integration-free methods under selection?

No, it is not necessary to maintain integration-free, reprogrammed iPSCs under selection. However, since iPSCs have a natural tendency to differentiate, the cells require specific culture techniques that balance the promotion of pluripotent cell growth with the inhibition of spontaneous cellular differentiation. Please refer to the [ATCC® Stem Cell Culture Guide](#) found on the ATCC website.

5. Do you have a chemically defined media system for iPSCs and dissociation reagents?

ATCC does not offer chemically defined media or dissociation reagents at this time; however, our media system is serum-free and xeno-free.

6. Do you have products for iPSC differentiation, for example to study neurogenesis, chondrogenesis and osteogenesis?

We do not offer products specifically for iPSC differentiation. We do offer differentiation kits for the Mesenchymal Stem Cells, including adipocyte differentiation ([ATCC® No. PCS-500-050](#)), chondrocyte differentiation ([ATCC® No. PCS-500-051](#)), and osteocyte differentiation kits ([ATCC® No. PCS-500-052](#)).

7. Do you use standard cell culture methods, like flasks, for culturing iPSCs?

iPSCs can be grown either in tissue culture flasks or in tissue culture dishes. However, many researchers find tissue culture dishes more convenient for manipulating and removing unwanted differentiated iPSCs. It is necessary to either co-culture iPSCs with feeder layer or coat the tissue culture dishes with a substrate, such as CellMatrix Basement Membrane Gel ([ATCC® No. ACS-3035](#)). To view the complete list of available feeder cells visit www.atcc.org.

Mesenchymal Stem Cell Questions

8. Does ATCC have a complete CD characterization certificate for the mesenchymal stem cells?

We assay the MSCs for positive expression of CD29, CD44, CD73, CD90, CD105 and CD166 and negative expression of CD14, CD31, CD34, CD45, using cell specific staining. The results are reported on the lot specific *Certificate of Analysis* for the MSC.

9. How long does chondrocyte differentiation take?

When the ATCC Chondrocyte Kit ([ATCC® No. PCS-500-051](#)) is used, it takes approximately 21 days to complete the differentiation protocol.

10. Do you have a recommended staining method for monitoring osteogenic differentiation?

To confirm calcium accumulation after osteogenic differentiation, you can fix the cells and stain them with Alizarin Red. Calcium forms an Alizarin Red – calcium complex in a chelation process.

11. Will MSC differentiation media be available to differentiate cells into fibroblasts or keratinocytes?

At this time, ATCC does not plan to offer MSC differentiation media for fibroblast or keratinocyte differentiation.

Passaging, Freezing and Media Questions

12. Why is it difficult to make home brew serum-free, feeder-free media?

Serum-free, feeder-free medium is prepared using supplements that may vary in potency and/or activity from lot-to-lot. As a result, one batch of a given defined medium may support growth, pluripotency and/or differentiation capacity better than another. Each time you prepare a batch of a medium, it must be carefully tested to ensure that the batch supports growth, pluripotency and differentiation capacity as well as previous batches. This validation process is time and reagent consuming, but is a critical step that cannot be eliminated. In addition, the supplements used to prepare these media are expensive. When you purchase a commercially prepared serum-free, feeder-free medium, all of the validation work has been done for you. You can be assured the media will perform consistently and reliably from bottle to bottle.

13. Have you compared your media with other commercial media?

Yes, we have compared ATCC serum-free, feeder-dependent PSC SFM XF media ([ATCC® No. ACS-3001](#)) and serum-free, feeder-free PSC SFM XF/FF media ([ATCC® No. ACS-3002](#)) to several other commercial media. We found our media to perform equally well or better in some cases.

14. What are the components in your stem cell freezing media? How does it ensure better cryopreservation than glycerol or DMSO? Do you have freezing media for tumor cells, too?

The formulation of ATCC Stem Cell Freezing Medium ([ATCC® No. ACS-3020](#)) is proprietary. It is a serum-free, xeno-free, ready to use complete cryopreservation medium. We also offer a freezing medium for use with continuous cell lines, including tumor cell lines. The ATCC Serum Free Cell Freezing Medium ([ATCC® No. 30-2600](#)) contains 10% DMSO and methylcellulose, however the complete formulation is also proprietary.

15. Is it always necessary to use a Rock Inhibitor?

It is not always necessary to use a ROCK (Rho-associated kinase p160ROCK) inhibitor. However, there are definite benefits to using a ROCK inhibitor. For example, treatment with a ROCK inhibitor helps prevent apoptosis of iPSCs due to manipulations during dissociation. It also helps enhance the survival rate of stem cells during cryopreservation and recovery from cryopreservation.

16. What is the recommended protocol for freezing ESCs?

The recommended protocol for freezing ESCs can be found in the [ATCC Stem Cell Culture Guide](#).

17. Can cancer stem cells be cultured in petri dishes?

Generally, petri dishes are not recommended for culturing animal cell lines. Petri dishes are non-treated plastic dishes that may not support cell adherence. It is possible to use tissue-culture treated cell culture dishes to culture cancer stem cells.

18. Do you have any information about passaging stem cells by automated mechanical passaging (i.e. Chopper)? In particular, do you recommend this method for passaging neurospheres?

We do not utilize automated mechanical passaging at this time.