StemScale PSC suspension culture protocol guide

Frequently asked questions and troubleshooting

Contents

Preparation and storage

1. **How should I store complete Gibco™ StemScale™ PSC Suspension Medium?**
   Following reconstitution, the complete medium can be stored at 2°C–8°C for up to 2 weeks.

2. **Can I aliquot and freeze Gibco™ StemScale™ PSC Suspension Supplement?**
   Yes. StemScale supplement may be aliquoted into smaller volumes upon receipt and stored at –20°C to –50°C for up to 6 months. Avoid multiple freeze-thaw cycles.

3. **Can I aliquot and freeze complete StemScale PSC Suspension Medium?**
   Yes. If the entire 1 L of **complete** StemScale PSC Suspension Medium will not be consumed within 2 weeks, the medium may be aliquoted into smaller volumes and stored at –5°C to –20°C for up to 6 months.

4. **Can the frozen aliquots of complete StemScale PSC Suspension Medium and StemScale PSC Suspension Supplement undergo multiple freeze-thaw cycles?**
   No. To ensure optimal performance, each aliquot of complete StemScale medium and StemScale supplement should undergo only one freeze-thaw cycle. Once an aliquot is thawed, it should not be frozen a second time.

5. **Will freezing complete StemScale PSC Suspension Medium or StemScale PSC Suspension Supplement affect performance?**
   No. Aliquots of complete StemScale medium and StemScale supplement show no significant decrease in performance after one freeze-thaw cycle. Multiple freeze-thaw cycles, however, are not recommended.

Protocol recommendations

6. **How many components does the StemScale PSC Suspension Medium kit consist of?**
   The StemScale PSC Suspension Medium kit consists of two components: a basal medium and a supplement. Following reconstitution, the complete medium is used for all steps, from initiating cultures to feeding cultures.

7. **How can I prevent the spheroids in my suspension cultures from aggregating into large clumps when I remove the cultures from the shaker platform?**
   When suspension cultures are removed from agitation, spheroids may begin to aggregate into large clumps. However, aggregation into clumps will not occur immediately. To avoid undesirable aggregation, the time that the suspension cultures are agitated in the shaker platform should be minimized to no longer than 15 minutes.

8. **Do you have any recommendations for handling suspension cultures when imaging?**
   When placed into a 6-well plate, the suspension cultures can be gently swirled in a clockwise motion to draw spheroids toward the center of the well. Images should then be acquired as quickly as possible to avoid undesirable spheroid aggregation. After acquiring images, gently agitate the plate to disperse spheroids throughout the well.

9. **How can I acquire images of suspension cultures that are not already growing in a well-plate format?**
   For suspension cultures that are growing in shaker flasks or spinner flasks, swirl flasks to ensure even distribution of cells and then remove a small volume of medium (~2–3 mL) containing spheroids from the flask. This aliquot of the suspension culture can be placed into a 6-well plate for imaging.

10. **Will the time required for gravity sedimentation cause my spheroids to clump?**
    The amount of time the spheroids settle via gravity sedimentation should be minimized to avoid undesirable spheroid aggregation. The 5-minute gravity sedimentation recommended in the feeding protocol has no adverse effect on spheroids.

11. **Do I need to wait the full 5-minute duration for gravity sedimentation to occur?**
    When spheroids are small and not easily visible by eye (i.e., day 1–2 of culture), do not allow spheroids to settle for more than 5 minutes during feeding. Smaller spheroids are more likely to aggregate into clumps if allowed to settle for longer than 5 minutes.

    When spheroids are large and easily visible by eye (i.e., day 3–day 5 of culture), the spheroids do not require the full 5 minutes of gravity sedimentation to collect at the bottom of the well. The medium can be replaced once the user can visually confirm all spheroids have been collected at the bottom of the well.

12. **How can I control the size of my spheroids?**
    The recommended method for controlling the size of spheroids is by changing the rotor speed (RPM). When initiating cultures, a higher RPM will lead to a reduced spheroid diameter expansion rate relative to cultures initiated at a lower RPM.

    Consequently, spheroids will appear smaller at the end of the culture period in cultures incubated at higher RPM.

    Changing the culture volume is another method that can influence spheroid size for orbital shaker platform cultures. When initiating cultures, a larger volume will lead to an increased spheroid diameter expansion rate relative to cultures with lower volumes.

    Consequently, spheroids will appear larger at the end of the culture period in the large-volume cultures. An increased volume method alone is not optimal as bigger spheroids tend to fuse at the end of the culture period.

13. **Will spheroids be more uniform in size at higher RPM, given the same volume of medium?**
    Yes. While spheroids will be smaller at higher RPM, they will also be more uniform in size.

14. **If I do not have the Thermo Scientific™ CO_2 Resistant Shaker, how will this affect the RPM settings?**
    The RPM may need to be altered for other orbital shaker platforms, depending on the orbital diameter. The diameter of the orbital shaker will affect the shear force. It is possible to estimate the RPM for another platform by using the following equation:

$$RPM_2 = \sqrt{(RPM_1{}^2 \times \frac{d_1}{d_2})}$$

    Where d_1 = orbital diameter of the Thermo Scientific CO_2 Resistant Shaker (0.75 in.); d_2 = orbital diameter of the alternative orbital shaker platform; RPM_1 = recommended RPM for the Thermo Scientific CO_2 Resistant Shaker; RPM_2 = recommended RPM for the alternative orbital shaker platform.

15. **How do I obtain greater numbers of spheroids in the same culture vessel?**
    A greater number of spheroids can be obtained by simply increasing the initial seeding density. Increasing the seeding to very high densities (e.g., >300,000 cells/mL) is not recommended, as medium will be consumed far more rapidly than at the recommended seeding density. Consequently, necrotic cores may develop more easily at higher seeding densities.

    Alternatively, instead of manipulating the seeding density, both RPM and culture volume can be simultaneously modified for orbital shaker platform cultures. By increasing both RPM and culture volume simultaneously, the number of cells used to seed a suspension culture can be increased while maintaining the recommended 150,000 cells/mL seeding density. Consequently, a greater number of spheroids can be obtained from the same culture vessel.

Example using a 125 mL shaker flask:

- Standard recommended conditions are: 70 RPM and a 20 mL culture volume
- To obtain greater numbers of spheroids: 90 RPM and a 40 mL culture volume

For shaker flasks, increasing the shaker speed from 70 RPM to 90 RPM necessitates an approximate doubling of the culture volume.

16. **How do I clean glass vessels for suspension cultures?**
    The steps for the recommended cleaning procedure are described below:

    - Glass vessels should first be soaked in a 1% solution of 7X detergent, either for 2–3 hr or overnight.
    - After removing the detergent solution, glass vessels should be scrubbed lightly with a brush and rinsed 3–4 times with tap water. Vessels should air dry completely in preparation for the next step.
    - Once the detergent cleaning has been completed, glass vessels must be siliconized to prevent cells from adhering to their surfaces. Addition of Thermo Scientific™ Water-Soluble Siliconizing Fluid for 5 minutes is sufficient to induce siliconization.
    - After removal of Thermo Scientific Water-Soluble Siliconizing Fluid, rinse vessels 3–4 times with tap water and air dry.
    - Place glass vessels into sterilization bags and autoclave to ensure glass vessels remain sterile for future experiments.

17. **I routinely use Gibco™ RevitaCell™ Supplement for applications with adherent cultures. To initiate suspension cultures, can I also use RevitaCell Supplement instead of ROCK inhibitor (Y-27632, Sigma Aldrich) when preparing 6-well plates or shaker flasks with StemScale PSC Suspension Medium?**
    Continue to use RevitaCell Supplement for applications with your **adherent** cultures. For optimal spheroid nucleation in suspension culture, however, we specially recommend ROCK inhibitor (Y-27632).

18. **How confluent should my adherent cultures be before initiating my suspension culture?**
    For best results, your adherent cultures should be 70–80% confluent. The overall growth and expansion of your suspension cultures may decrease if overly confluent adherent cultures are used for seeding.

19. **How many cells in total are needed to seed an entire 6-well plate?**
    Using our recommended seeding density of 150,000 cells/mL, a total of 1.8×10^6 viable cells is necessary to seed an entire 6-well plate for suspension culture.

20. **What is the minimum final volume of medium necessary in each well when seeding a 6-well plate?**
The recommended final volume of medium when seeding a 6-well plate is 2 mL per well at 70 RPM. When seeding at 70 RPM, volumes less than 1.5 mL or more than 2.5 mL are not recommended.

21. **What changes do I need to make to the protocol if I am using well plates smaller than a 6-well format?**
When using well plates smaller than a 6-well format to grow suspension cultures, the culture volume and RPM will need to be adjusted to account for the smaller well size. In general, the culture volume will decrease while the RPM will increase.

The table below shows recommendations for other well plate formats:

Vessel format	Culture volume	Rotation
6-well plate	2 mL per well	70–80 RPM
12-well plate	1 mL per well	90–100 RPM
24-well plate	500 µL per well	120–130 RPM
48-well plate	250 µL per well	150–160 RPM

22. **The protocol includes the use of Gibco™ StemPro™ Accutase™ reagent for passaging suspension cultures. Can dissociation medium other than StemPro Accutase reagent be used when passaging?**
Gibco™ TrypLE™ Select enzyme may be used instead of the StemPro Accutase reagent, but only if DNase is added with ROCK inhibitor during cell seeding. The recommendation is to use 1 unit of DNase per 1 mL of medium.

Gibco™ Versene™ Solution (EDTA) is not recommended for routine suspension culture passaging. Versene Solution is intended for clump passaging, and consequently does not effectively dissociate spheroids.

23. **During passaging, is it necessary to wash with PBS wash prior to addition of StemPro Accutase reagent?**
No. Washing with PBS before adding StemPro Accutase reagent is not necessary.

24. **How do I determine the final cell concentration using a hemocytometer?**
The equation for a hemocytometer is as follows:
Final cell concentration = (average cell count from one corner square) x (dilution factor) x (10,000)

25. **When imaging spheroids, will I be able to see indications of necrosis or estimate the size of necrotic cores?**
While it can be difficult to estimate the presence of a necrotic core, a good indication is the coloration of the spheroid. Spheroids become darker in color as they grow and begin to form a necrotic core. We recommend passaging before the average spheroid diameter of 400 µm is exceeded.

Large spheroids that appear dark are not unusual and should be passaged as they are likely at the point of beginning to develop a necrotic core.
Small spheroids that appear dark are likely to be experiencing stress and may require more frequent medium exchanges or a lower initial seeding density.

26. **What volume of StemPro Accutase reagent should I use, and how long should I incubate the spheroids in StemPro Accutase reagent at 37°C?**
The volume of StemPro Accutase reagent and incubation time will vary depending on the culture size and vessel. In general, larger vessels with greater numbers of spheroids will require higher volumes of StemPro Accutase reagent and longer incubation times. Guidance is provided in the following table. Incubation times can vary and may be extended for longer than listed in the table if the spheroids are not completely dissociated.

Vessel format	StemPro Accutase reagent volume	StemPro Accutase reagent incubation time
6-well plate	1 mL per well	10 min
24-well plate	1 mL per well	10 min
125 mL shaker flask	5 mL per flask	10–15 min
250 mL shaker flask	10 mL per flask	15 min
500 mL shaker flask	20 mL per flask	15–20 min
100 mL bioreactor	15 mL per flask	20 min
500 mL bioreactor	50 mL per flask	20 min

27. **During passaging, one step involves centrifuging the spheroids. Can I also spin down the spheroids during routine feeding?**
No. The spheroids are centrifuged only during passaging and should not be centrifuged during routine feeding.

28. **During passaging, is it necessary to place the conical tube with the spheroids in a water bath or incubate at 37˚C after adding StemPro Accutase reagent?**
Yes. Dissociation with StemPro Accutase medium occurs faster at 37°C, which helps break apart spheroids more rapidly.

29. **How do I assess the degree of spheroid dissociation during StemPro Accutase reagent incubation? How do I know if dissociation is complete?**
Dissociation can be assessed by visual inspection. The longer the spheroids are exposed to StemPro Accutase reagent, the more easily they will break apart with gentle agitation of the conical tube; floating clumps should not be present. Dissociation is complete when the suspension in the conical tube is homogeneous and forms a cloudy single-cell suspension.

30. **How should I passage my cultures that are larger than 20 mL? Should I use multiple 50 mL conical tubes during StemPro Accutase reagent dissociation and cell counting?**
Multiple 50 mL conical tubes may be required for large-scale cultures. Large-volume conical tubes (i.e., 250 mL) can also be utilized if your centrifuge is able to accommodate these larger conical tubes.

    Once the 50 mL conical tubes containing the spheroids have been centrifuged, we recommend combining all the spheroids into a single conical tube prior to dissociation with StemPro Accutase reagent. This will reduce hands-on time during dissociation. However, multiple conical tubes can be used during dissociation if preferred by the user. For cell counting, the entire dissociated cell suspension should be combined into a single conical prior to centrifugation.

31. **Does the StemScale passaging protocol require a cell strainer to dissociate spheroids?**
No. The StemScale passaging protocol does not require a cell strainer, as spheroids are sufficiently dissociated into single cells through exposure to StemPro Accutase reagent.

32. **How many 6-well plates or culture vessels can I feed at the same time?**
When first growing suspension cultures in well plates or other culture vessels, we recommend feeding these vessels one at a time. As users become more experienced and familiar with the performance of their cell lines in suspension, they can choose to increase the number of cultures that are fed simultaneously. Experienced users can become comfortable feeding multiple 6-well plates or 125 mL shaker flasks simultaneously.

33. **When feeding 6-well plates, how much medium should I remove from each well?**
Removing 50% of the medium from each well and replacing it with fresh medium is recommended. Depending on the cell line, or if the seeding density was higher than recommended, users may choose to replace more than 50% of the medium.

34. **When feeding shaker flasks, can I swirl the shaker flask before letting the spheroids settle to the bottom of the flask?**
Yes. Swirling the shaker flask before letting the spheroids settle can help collect the spheroids in the center at the bottom of the flask. This placement may allow for easier removal of medium. However, be sure that the shaker flask is not swirled after the spheroids have settled to the bottom and before removing 50% of the medium.

35. **Do you have any helpful tips for feeding my suspension cultures in shaker flasks?**
Yes. The suggestions listed below may be helpful when feeding your suspension cultures:

    - Loosen the cap on the shaker flask before preparing your serological pipet so that the cap can be easily removed with one hand.
    - After removing the spent StemScale medium, be sure to add the new StemScale medium down the side of the flask to avoid dispensing directly on top of the spheroids settled at the bottom of the flask. This technique helps prevent the formation of bubbles in the medium.

36. **How can I tell if my PSC spheroids are differentiating in culture?**
While it can be difficult to determine the extent of differentiation in suspension cultures, there are strong visual signs that may indicate that PSC spheroids are differentiating. PSC spheroids are likely to lose their rounded shape, and pronounced cavitations are likely to be visible at the onset of differentiation. We recommend passaging suspension cultures when the average spheroid diameter is 400 µm to avoid differentiation of spheroids grown in StemScale PSC Suspension Medium.

Performance

37. **Does StemScale PSC Suspension Medium maintain the pluripotency of hPSCs in suspension culture?**
Yes. StemScale medium maintains the pluripotency of hPSCs grown as spheroids in suspension culture for over 30 consecutive passages as assessed by both flow cytometric analysis and the PluriTest™ Assay.

38. **Will single-cell dissociation of the PSC suspension spheroids, with StemPro Accutase reagent, affect pluripotency or cause mutations in the long term?**
No. Spheroids grown in StemScale PSC Suspension Medium that are dissociated with StemPro Accutase reagent do not have altered pluripotency or karyotypes, even after high passage numbers (>30 passages), as assessed by flow cytometric analysis, the PluriTest Assay, or the Applied Biosystems™ KaryoStat™ Assay.

39. **Does StemScale PSC Suspension Medium maintain normal karyotypes of hPSCs in suspension culture?**
Yes. StemScale medium maintains normal karyotypes of hPSCs grown as spheroids in suspension culture for over 30 consecutive passages as assessed by the KaryoStat Assay.

40. **Is StemScale PSC Suspension Medium able to maintain high viability of human PSCs (hPSCs) in suspension culture?**
Yes. Gibco™ Trypan Blue staining of single cells obtained from dissociated spheroids indicates that the viable cell count of suspension cultures remains high (>90%).

41. **Does StemScale PSC Suspension Medium maintain trilineage differentiation potential of hPSCs in suspension culture?**
Yes. StemScale medium maintains trilineage differentiation potential of hPSCs as assessed by the Applied Biosystems™ TaqMan® hPSC Scorecard™ kit.

42. **Is StemScale PSC Suspension Medium compatible with multiple different cell lines?**
Yes. We have evaluated over 10 different induced PSC (iPSC) and embryonic stem cell (ESC) lines, all of which were demonstrated to be compatible with StemScale medium.

43. **What vessels are suitable for my suspension cultures grown in StemScale PSC Suspension Medium?**
A variety of culture vessels, including non–tissue culture treated well plates, plain-bottom shaker flasks, and bioreactors, are suitable for suspension cultures grown in StemScale PSC Suspension Medium.

44. **Does complete StemScale medium require the use of microcarriers to form spheroids?**
No. StemScale medium promotes the formation of spheroids through self-aggregation.

45. **Spheroids form on day 1 after seeding in StemScale PSC Suspension Medium, but they are not perfectly round. Is this normal?**
Yes. If suspension cultures are grown using an orbital shaker platform, spheroids that are not perfectly round on day 1 may be observed. By day 2, these spheroids will take on a more uniform, rounded shape.

46. **What is the fold expansion per passage that I can expect using StemScale PSC Suspension Medium?**
On average we have observed greater than 4-fold expansion per passage, but this can vary by cell line. Some cell lines can reach up to 10X expansion.

47. **What is the recommended passaging schedule when using StemScale PSC Suspension Medium?**
The StemScale PSC Suspension Medium protocol recommends passaging every 4–5 days, depending on spheroid size. We recommend passaging suspension cultures when the average spheroid diameter is 400 µm.

    Passaging cultures on day 3 is also an option but may result in a lower cell yield. Day 3 passaging can be performed to avoid passaging on a weekend.

48. **Do I need to feed my cultures daily with StemScale PSC Suspension Medium?**
No. The StemScale medium feeding schedule allows for every-other-day feeding. While suspension cultures can be fed daily to maximize cell health, StemScale medium allows users the flexibility to feed suspension cultures every other day without sacrificing performance.

    Weekend-free feeding is also possible by skipping two days in a row. Although weekend-free cultures exhibit reduced expansion compared to daily and every-other-day cultures, the viability and pluripotency of weekend-free cultures still remains high.

49. **Should I be concerned about waste by-products accumulating in my suspension cultures using the StemScale PSC Suspension Medium feeding method?**
No. The StemScale PSC Suspension Medium feeding method involves replacing 50% of the spent medium with fresh medium. This feeding method prevents spheroids from being cultured in a medium that is accumulating significant quantities of waste by-products.

50. **Can I thaw frozen cells directly into StemScale PSC Suspension Medium?**
Yes. Cells that have been cryopreserved as single cells can be thawed directly into StemScale medium. The same protocol for thawing cells for adherent cultures can be utilized for suspension cultures. Once cell counts have been obtained, the thawed cells can then be seeded into suspension cultures using the recommended seeding conditions for StemScale medium.

    Users should expect cells to require one passage in suspension before recovering from cryopreservation. After this recovery passage, suspension cultures will perform similarly to those seeded from adherent cultures.

51. **Can I thaw a frozen cell stock that has TrypLE Select enzyme directly into StemScale PSC Suspension Medium?**
Yes. Cells passaged using either StemPro Accutase reagent or TrypLE enzyme can be seeded directly into StemScale medium. The use of DNase is not required for cells passaged using TrypLE enzyme and recovering from cryopreservation.

52. **Can I freeze my spheroids?**
No. We recommend that suspension cultures be cryopreserved as single cells.

53. **Spheroids are not forming. What should I do?**
First, confirm that ROCK inhibitor (Y-27623) was added to the culture on day 0. Spheroids will not form without the presence of a ROCK inhibitor. If a ROCK inhibitor was added, then reduce the RPM applied to the culture vessel. Spheroids are unable to form if the RPM is too high.

Troubleshooting

54. **The spheroids are forming large clumps. Is there anything I can do?**
First, confirm that non–tissue culture treated vessels, which prevent spheroids from adhering to the bottom of the vessels, are being used.

    If spheroids are forming large clumps in non–tissue culture treated vessels, then the RPM applied to the culture vessel may be too low. Increasing the RPM will prevent spheroids from aggregating into large clumps.

    Alternatively, for orbital shaker platform cultures, the volume of medium in the culture vessel may be too large for the applied RPM, preventing spheroids from remaining in suspension. Decreasing the volume of the medium will prevent spheroids from aggregating into large clumps.

55. **The spheroids were growing well but have suddenly formed large clumps. How can this be prevented?**
First, confirm that the suspension cultures have not been removed from agitation for extended periods of time. When imaging spheroids, cultures must not be removed from agitation for more than 15 minutes.

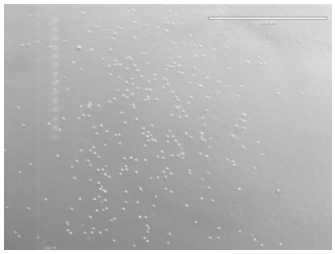
    For orbital shaker platform cultures, confirm that the culture volume has not increased since day 0. Increasing the culture volume makes it more difficult for spheroids to be sufficiently agitated. If the culture volume has drifted, spheroids may no longer remain suspended at the current RPM.

Confirm that the orbital shaker platform was constantly powered. A power outage may have occurred and interrupted agitation temporarily. Spheroids require constant agitation to grow properly. Having the orbital shaker platform connected to a battery backup will ensure that the cultures will be constantly agitated even in the event of a power outage.

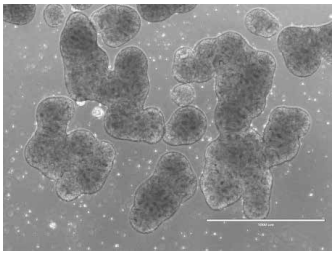
56. **Why is there less medium in my culture even if I am adding back the same volume that I remove when feeding?**
A lower volume of medium may be a result of evaporation. To help minimize evaporation, keep the incubator water pan full.

57. **What do the suspension cultures look like:**

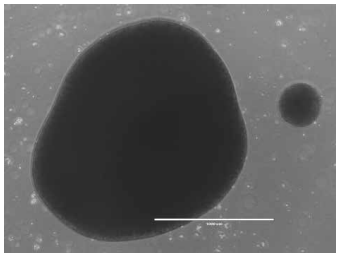
a) If I did not add ROCK inhibitor (Y-27632) at day 0?
Spheroids will not form without the presence of a ROCK inhibitor on day 0.



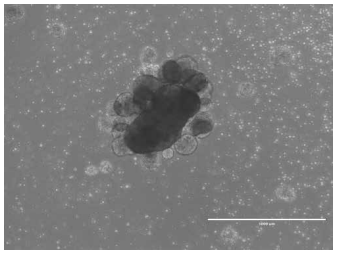
b) If I have removed my cultures from agitation for an extended time period?
Spheroids will attempt to aggregate into large clumps. The image below illustrates how individual spheroids are beginning to form large clusters of spheroids.



c) If the volume of medium in my culture is too high or the RPM is too low?
If the cells in the culture vessel are not sufficiently agitated, they will all aggregate into a large clump.



d) If I used TrypLE Select enzyme without including DNase?
Without DNase, the presence of small quantities of lysed DNA due to dissociation by TrypLE Select enzyme will cause some spheroids to aggregate into a single clump.



Comparison to other commercially available products

58. **Managing multiple components in a medium kit can be challenging. Does the StemScale PSC Suspension Medium kit have a similar number of components as others?**
The StemScale PSC Suspension Medium kit consists of two components: a basal medium and a supplement. Following reconstitution, the complete medium is used for all steps, from initiating cultures to feeding cultures.

Other suspension culture medium kits sometimes consist of multiple components, which may be utilized at different points in the suspension culture process.

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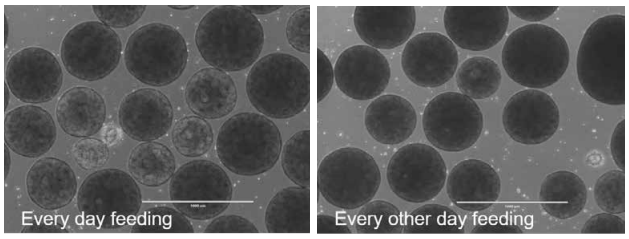
59. **How does the StemScale feeding schedule differ from that of other suspension culture media?**
While other suspension culture media may require daily feeding of suspension cultures, the StemScale feeding schedule allows for every-other-day feeding.

    With StemScale medium, cultures can be fed daily to maximize cell health. However, users have the option to feed every other day without sacrificing performance while also providing more flexibility in the suspension culture schedule.



60. **Are there advantages to the StemScale feeding method as compared to those for other suspension culture media?**
The StemScale medium feeding method involves replacing 50% of the spent medium with fresh medium. This feeding method prevents spheroids from being cultured in a medium that is accumulating significant quantities of waste by-products.

    Feeding methods that use a fed-batch or overlay strategy do not remove the spent medium from suspension cultures. This method reduces hands-on time when feeding cultures; however, the accumulation of waste by-products is likely to negatively impact the health of these cells.

    The StemScale medium feeding strategy also ensures a consistent volume through the growth period of the PSC spheroids. This is in contrast to batch-feeding methods where the volume increases incrementally. Since culture volume is one of the determinants of shear force and spheroid size, the 50% feeding strategy maintains this variable constant throughout the growth period.

61. **How does the StemScale passaging protocol compare to protocols for other suspension culture media?**
The StemScale medium protocol recommends passaging every 4–5 days, depending on spheroid size. We recommend passaging suspension cultures when the average spheroid diameter is 400 µm, which occurs approximately after 4–5 days of growth. Passaging cultures on day 3 is also an option but may result in a lower cell yield. Day 3 passaging can be performed to avoid passaging on a weekend.

    Passaging protocols for other suspension culture media may offer less flexibility, depending on whether they utilize a fed-batch or overlay strategy. Generally, these cultures require a strict passaging schedule with little room for flexibility on the weekend.

    The StemScale medium passaging protocol does not require the use of microcarriers or cell strainers, while other PSC suspension culture medium protocols do.

Find out more at **thermofisher.com/stemscale**

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