Essential 8 Flex Medium

Frequently asked questions

Overview

Gibco™ Essential 8™ Flex Medium is based upon the defined Gibco™ Essential 8™ Medium formulation developed in the laboratory of James Thomson and validated by Cellular Dynamics International. Essential 8 Flex Medium is a serum-free, xeno-free medium that supports the culture and expansion of pluripotent stem cells (PSCs) without the need for daily feeding. This allows for weekend-free maintenance and expansion of PSCs without negatively impacting the health and density of the cultures and without substantial protocol adjustments.

Comparison to the original Essential 8 Medium

1. How does Essential 8 Flex Medium work without daily feeding?

Essential 8 Flex Medium has been reformulated from the original Essential 8 Medium to extend the activity of key heat-sensitive components found in PSC medium, including FGF2 (Figure 1).

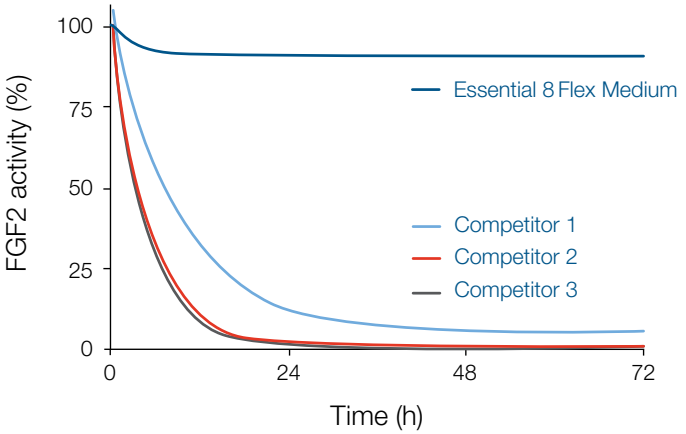

Figure 1. FGF2 activity over time in PSC culture medium. Unlike other feeder-free PSC culture media, Essential 8 Flex Medium has been optimized to extend the activity of unstable components such as FGF2. Extended activity allows for routine culture without the daily feeding.

2. How has original Essential 8 Medium formulation been modified to enable a more flexible culture schedule?

The original, defined Essential 8 Medium formulation has been optimized to allow for an extended period of time between PSC culture feedings.

3. Is the product configuration different than Essential 8 Medium?

No, the Essential 8 Flex Medium product configuration is the same as that of Essential 8 Medium: a basal medium with a frozen 50X supplement.

Protocol recommendations

4. How many days a week can I skip feeding my culture?

You are able to skip feeding up to two consecutive days (e.g., Saturday and Sunday), and up to three days total per week.

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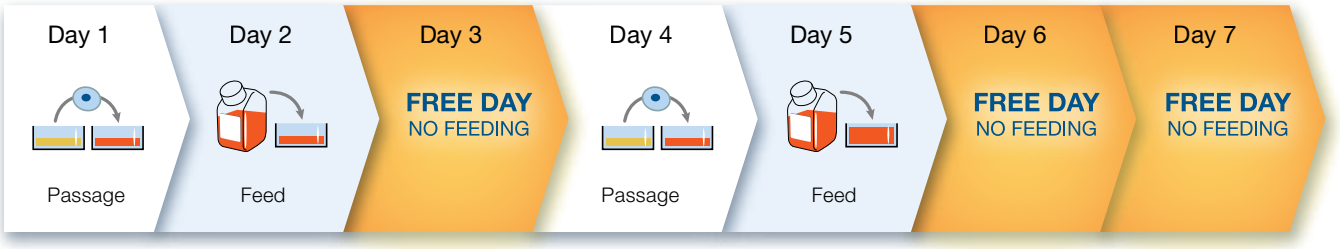

Figure 2. Weekend-free feeding protocol. Cells are split on Monday and Thursday (day 1 and day 4) with full media exchanges on Tuesday and Friday (day 2 and day 5, approximately 24 h after plating). The Friday (day 5) feed should use twice the standard volume.

5. How flexible is the Essential 8 Flex Medium feeding schedule? Can you give an example feeding schedule?

Best results are observed when cells are split twice weekly (Figure 2). To enable a weekend-free culture schedule, cells would be split on Monday (day 1) and Thursday (day 4) with full media exchanges on Tuesday (day 2) and Friday (day 5), approximately 24 h after plating. In this example, the Friday (day 5) feed should use twice the standard volume.

6. When should I start feeding my cells with Essential 8 Flex Medium?

Best results are observed when cultures are switched into Essential 8 Flex Medium at the start of the week and Essential 8 Flex Medium is routinely used in place of existing feeder-free culture systems.

7. How does Essential 8 Flex Medium differ from other weekend-free PSC culture solutions?

Unlike other commercially available PSC culture solutions that also offer weekend-free feeding, Essential 8 Flex Medium does not require substantial split ratio adjustments prior to the break in feeding. With Essential 8 Flex Medium, you can maintain your twice-weekly split schedule and skip weekend feeds.

8. Do you still recommend using vitronectin as the matrix for this culture system?

Both Gibco™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. No. A14700) and Gibco™ Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix (Cat. No. A1413301) have been used successfully with Essential 8 Flex Medium.

9. Is Gibco™ Versene Solution still the recommended dissociation reagent?

Both Versene Solution (Cat. No. 15040066) and enzyme-mediated dissociation have been shown to be compatible passaging methods.

10. What other dissociation reagents have been tested?

Gibco™ TrypLE™ Select Enzyme (Cat. No. 12563011) and Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent (Cat. No. A1110501) have been tested with Essential 8 Flex Medium.

11. Do I need to add a ROCK inhibitor during passaging?

As with many other PSC media, ROCK inhibitor is not required with Essential 8 Flex Medium when Versene Solution is used to passage PSCs as clusters. If cells are plated as singlets, we suggest that Gibco™ RevitaCell™ Supplement (Cat. No. A2644501) or another ROCK inhibitor be used.

12. What are the recommended passaging ratios?

Passaging ratios depend on the cell line you are using. Split ratios between 1:3 and 1:12 are generally well tolerated in Essential 8 Flex Medium.

13. What is the timing for medium changes and passaging?

Optimal results are observed when the cells are split twice weekly with a medium exchange approximately 24 h after plating. Splitting Monday (day 1) and Thursday (day 4) with Tuesday (day 2) and Friday (day 5, double volume) feeds have shown the best results.

14. How does this change my current split schedule?

If you are currently splitting your cells twice weekly you should not need to dramatically alter your current split schedule. Best results are observed with twice weekly splits, generally Monday and Thursday, with full media exchanges approximately 24 h after plating. We also advise that the volume of the Friday feed is twice the standard feed volume.

15. **If I intend to transition from the original Essential 8 Medium to Essential 8 Flex Medium, will I need to prepare the complete medium any differently?**

    The preparation of Essential 8 Flex Medium is equivalent to that of the original Essential 8 Medium. The only difference is in the feeding schedule, with Essential 8 Flex Medium enabling a more flexible, 2-day break between required culture feeds.

16. **Can I use Essential 8™ Base Medium with Essential 8™ Flex Supplement?**

    Essential 8 Flex Medium is a complete kit, with a base medium and frozen supplement designed to be used together. Essential 8 Flex Medium components should not be mixed with Essential 8 Medium components.

17. **Can I thaw the Essential 8 Flex Supplement in a 37°C water bath?**

    No, do not thaw the Essential 8 Flex Supplement in a 37°C water bath. Best results are observed thawing at room temperature for approximately 1 hour. You may also thaw the Essential 8 Flex Supplement overnight at 2–8°C, a process that could result in small amounts of precipitation. The presence of precipitation should not adversely affect the performance of the medium.

18. **If I see precipitation when I thaw the Essential 8 Flex Supplement, what should I do?**

    The appearance of precipitate in the Essential 8 Flex Supplement is rare; however, if it is seen, it should not affect the performance of the medium. If you see precipitation in the supplement, keep it well mixed and add it to the Essential 8 Flex Basal Medium as you normally would.

19. **What is the shelf life of Essential 8 Flex Medium after supplementation?**

    Best results are observed when complete Essential 8 Flex Medium is used within 2 weeks of supplementation.

20. **Can I warm Essential 8 Flex Medium in a 37°C water bath for daily use?**

    No, do not warm Essential 8 Flex Medium at 37°C. Best results are achieved when the medium is allowed to slowly reach room temperature.

21. **Can I use ROCK inhibitors with Essential 8 Flex Medium?**

    Yes, Essential 8 Flex Medium has been used with ROCK inhibitors. We suggest using RevitaCell Supplement (Cat. No. A2644501), which has been specifically designed to minimize the impact of stress on PSCs.

22. **Can I perform single cell passaging with Essential 8 Flex Medium?**

    Yes, Essential 8 Flex Medium is compatible with single cell passaging. Whenever you are performing single cell passaging, ensure that you use RevitaCell Supplement (Cat. No. A2644501) or some other ROCK inhibitor.

23. **Can I use Essential 8 Flex Medium in my reprogramming and gene editing workflows?**

    The compatibility of Essential 8 Flex Medium with reprogramming or gene editing workflows has not been tested.

24. **Can I frequently change the feeding schedule with Essential 8 Flex Medium?**

    Best results are observed if you choose and maintain a regular split and feed schedule. For example, if you desire a weekend-free feeding schedule you should consistently split Monday/Thursday and feed Tuesday/Friday.

25. **Can I cryopreserve cells cultured in Essential 8 Flex Medium?**

    Cells cultured routinely in Essential 8 Flex Medium can be cryopreserved and revived just as they are in Essential 8 Medium.

Comparison to other commercially available products

26. **If I'm currently using Essential 8 Medium, can I easily transition to Essential 8 Flex Medium? Can I use it for just a weekend if I'm going to be away?**

    Best results are observed when you maintain your PSCs consistently in one medium. Transitioning from Essential 8 Medium to Essential 8 Flex Medium is as simple as seeding your cells into Essential 8 Flex Medium at the beginning of the week. Best results are achieved if the cells are split one time prior to the 2-day feed-free period.

27. **I already use an existing feeder-free medium but would love to take a weekend off from maintaining my cultures. Can I switch to Essential 8 Flex Medium for just a weekend?**

    Because optimal PSC culture results are achieved using a single PSC culture medium, we do not recommend routinely switching cells from one medium to another. Optimal results are achieved when Essential 8 Flex Medium is used and maintained as the primary culture system.

28. What is the difference between Essential 8 Flex Medium and a time-release FGF2 substitute?

Time-release products such as StemBeads™ FGF2 release FGF2 into the growth medium to counteract the loss of FGF2 activity over time, while Essential 8 Flex Medium prevents the loss of FGF2 activity from even happening.

29. How does the workflow for StemBeads FGF2 compare to the workflow for Essential 8 Flex Medium?

Unlike StemBeads FGF2, Essential 8 Flex Medium does not require any additional supplementation or optimization to limit the loss of FGF2 activity. (Source: StemBeads catalog web page.)

Performance

30. What is the long-term impact on my cultures after adopting a consistent feed schedule with consecutive feed-free days?

We have observed no impact from skipping daily feeding with Essential 8 Flex Medium for up to 50 passages in multiple cell lines. Pluripotency marker expression (Tra-1-60, SSEA4, Sox2, Oct-4, and Nanog), differentiation potential, and normal karyotypes are all maintained in long-term culture with Essential 8 Flex Medium.

31. Is there an effect of accumulated waste product buildup during my two-day feed-free period?

As with any PSC culture, some cell debris and pH drift will occur with Essential 8 Flex Medium. However, we have observed no adverse long-term effects from waste product buildup, either on cell growth or pluripotency.

32. Does routine culture in Essential 8 Flex Medium have any effect on the karyotype of my cells?

We have observed normal karyotypes in multiple PSC lines with long-term culture up to 50 passages.

33. Can I use PSCs that are long-term cultured in Essential 8 Flex Medium for downstream differentiation?

We have seen no effect on downstream differentiation potential for cells cultured in Essential 8 Flex Medium for up to 15 passages. Tri-lineage potential has been demonstrated from embryoid bodies as well as using Gibco™ PSC Neural Induction Medium (Cat. No. A1647801), Gibco™ PSC Cardiomyocyte Differentiation Kit (Cat. No. A25042SA), and Gibco™ PSC Definitive Endoderm Induction Kit (Cat. No. A27654SA).

34. Will I see a morphological difference between cells cultured in Essential 8 Medium and cells cultured in Essential 8 Flex Medium?

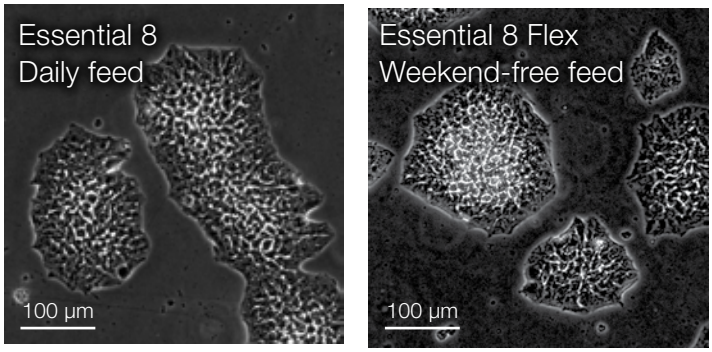

Figure 3. PSC morphology in Essential 8 Flex Medium. Standard PSC morphology is observed in long-term Essential 8 Flex Medium cultures without weekend feeding.

Cells cultured in Essential 8 Flex Medium maintain the expected PSC morphology, with compact, homogeneous colonies, defined edges, and a high nucleus-to-cytoplasm ratio (Figure 3).

35. Do you see any change in pluripotency marker expression from PSCs cultured in Essential 8 Flex Medium?

We have observed no significant change in pluripotency marker expression in multiple cell lines over 50 passages in Essential 8 Flex Medium.

36. Have you confirmed tri-lineage differentiation potential from PSCs cultured in Essential 8 Flex Medium?

The potential for cells from Essential 8 Flex Medium cultures to differentiate into cells of ectoderm, mesoderm, and endoderm lineages is unaffected by long-term culture in Essential 8 Flex Medium. This has been confirmed using both spontaneous differentiation from embryoid bodies and directed differentiation to neural stem cells, cardiomyocytes, and definitive endoderm cells.

Find out more at **thermofisher.com/essential8flex**

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