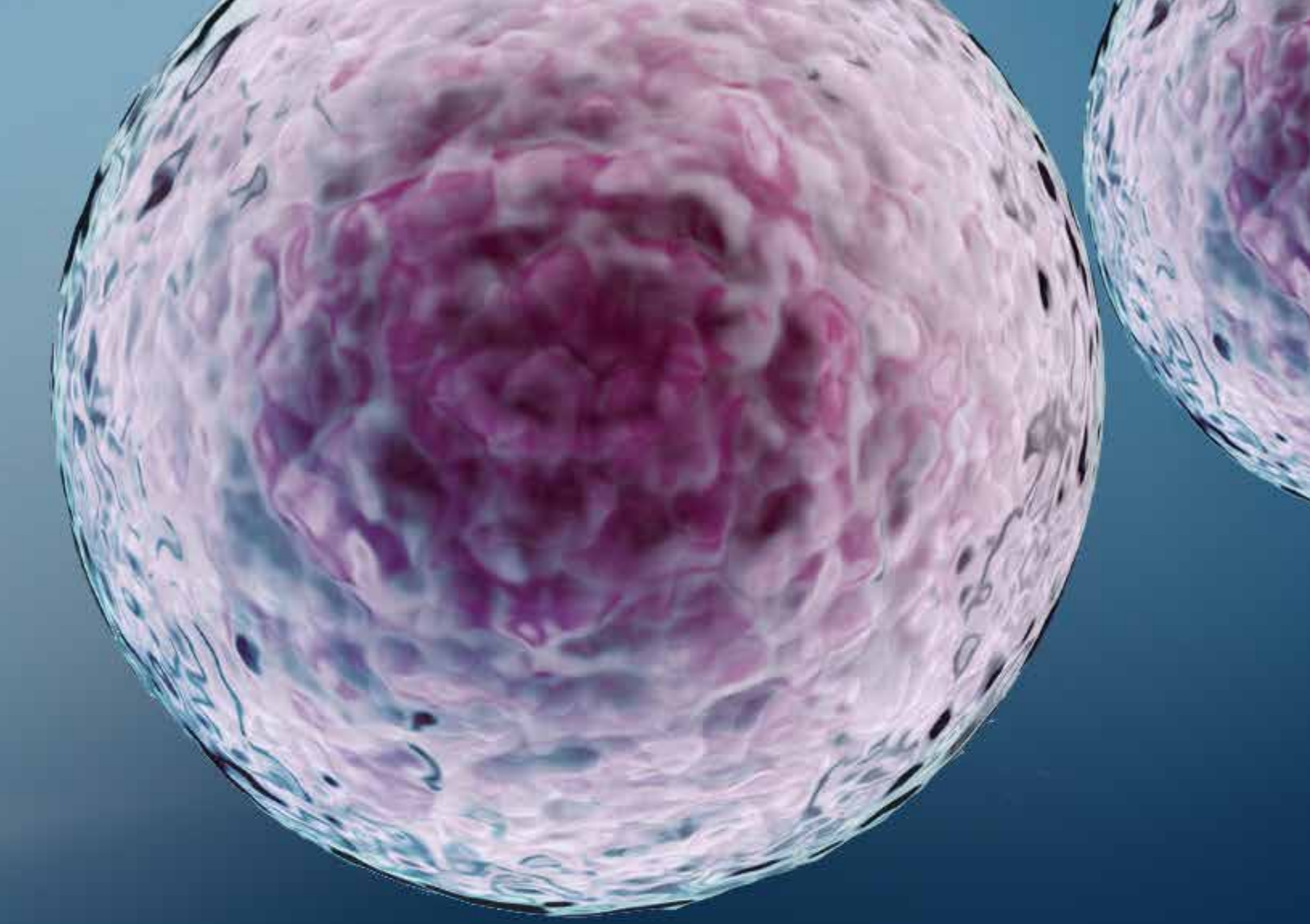
ThermoFisher SCIENTIFIC

STEMSCALE PSC SUSPENSION MEDIA

A new dimension to PSC culture

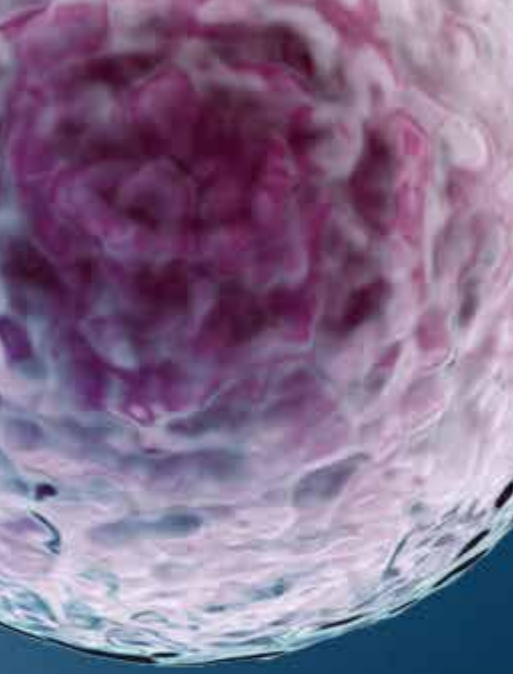
gibco



The value of suspension culture

As the use of pluripotent stem cells (PSCs) in research, drug discovery, and therapeutic applications continues to expand, the need to create large numbers of high-quality cells in a robust and cost-effective manner becomes ever more pressing. Media formulations that enable scalable culture and support efficient downstream differentiation while minimizing material, labor, and production requirements will greatly facilitate the use of PSCs for these applications. To address the challenges, researchers have adopted three-dimensional (3D) suspension culture systems that offer advantages for scale-up over two-dimensional (2D) adherent culture.

Notably, suspension cultures have a lower overall cost, reduced hands-on time and lab footprint, and greater compatibility with closed systems compared to adherent culture systems. Furthermore, suspension culture systems consume less media and plasticware than the same number of cells grown in adherent culture systems. For these reasons, suspension culture systems are more desirable for generating large quantities of PSCs. However, for applications requiring large numbers of cells, suspension culture systems need to provide high cell expansion capability, simplified protocols, and consistent performance across cell lines. In response to these challenges, we developed Gibco™ StemScale™ PSC Suspension Medium and Gibco™ CTS™ StemScale™ PSC Suspension Medium. Here, we describe how researchers can use these media to readily transition their existing adherent cultures to suspension cultures and realize a range of benefits compared to standard monolayer cultures.



Contents

StemScale PSC Suspension Medium

StemScale PSC Suspension Medium is a scalable, easy-to-use medium that supports robust expansion of PSCs in suspension, delivering 5- to 10-fold expansion per passage. It is specifically formulated to address the current technical challenges with PSC self-aggregation. StemScale medium promotes consistent spheroid formation and expansion capability across PSC lines and maintains pluripotency across multiple passages.

Key benefits

- **Superior expansion capability**—StemScale medium promotes a 5- to 10-fold expansion per passage and over three times the expansion capability compared to other PSC suspension media
- **Simplified workflow**—exchange media easily and initiate spheroid formation across multiple cell lines without the use of microcarriers or cell strainers during culture or passaging; choose to feed cultures every day or every other day, which provides more freedom when expanding PSCs
- **Scalable across formats**—StemScale medium is compatible with vessel sizes ranging from 6-well plates to medium-size bioreactors
- **Consistency across cell lines**—get reliable spheroid formation, consistent spheroid growth, and maintenance of sustained pluripotency and cell viability

CTS StemScale PSC Suspension Medium

CTS StemScale PSC Suspension Medium is designed to support expansion of PSCs at scale in suspension culture. Its scalable, easy-to-use workflow and xeno-free formulation make it an exceptional choice for translational and clinical applications.

Key benefits

- **Superior expansion capability**—achieve up to 30% higher fold expansion than with other PSC suspension media, reducing manufacturing time and cost
- **Scalable expansion for clinical applications**—grow PSCs across multiple culture vessel formats and sizes, from small to large scale
- **No cell strainer required**—remove scale limitation, reduce contamination risk and hands-on time, and improve cell viability and yield
- **Intended for cell therapy manufacturing***—streamline regulatory filings with GMP-manufacturing, extensive safety testing, traceability of raw materials, and regulatory documentation

Learn more at **thermofisher.com/ctsstemscale**

* Gibco™ CTS™ products are manufactured at a site that uses methods and controls that conform with CGMP for medical devices under 21 CFR Part 820. Our FDA-registered manufacturing site is ISO 13485-certified. Specific intended use statements and full documentation traceability are available and we offer convenient access through a letter of authorization to our Drug Master File (DMF) for the CTS StemScale PSC Suspension Medium.

Transition from research to clinic

CTS StemScale PSC Suspension Medium has a formulation similar to that of StemScale PSC Suspension Medium, Research Use Only (RUO), with specific modifications in line with regulatory guidance. With a few minor protocol differences (Table 1), CTS StemScale PSC Suspension Medium delivers similar performance, including cell yield, morphology, and pluripotency (Figure 1).

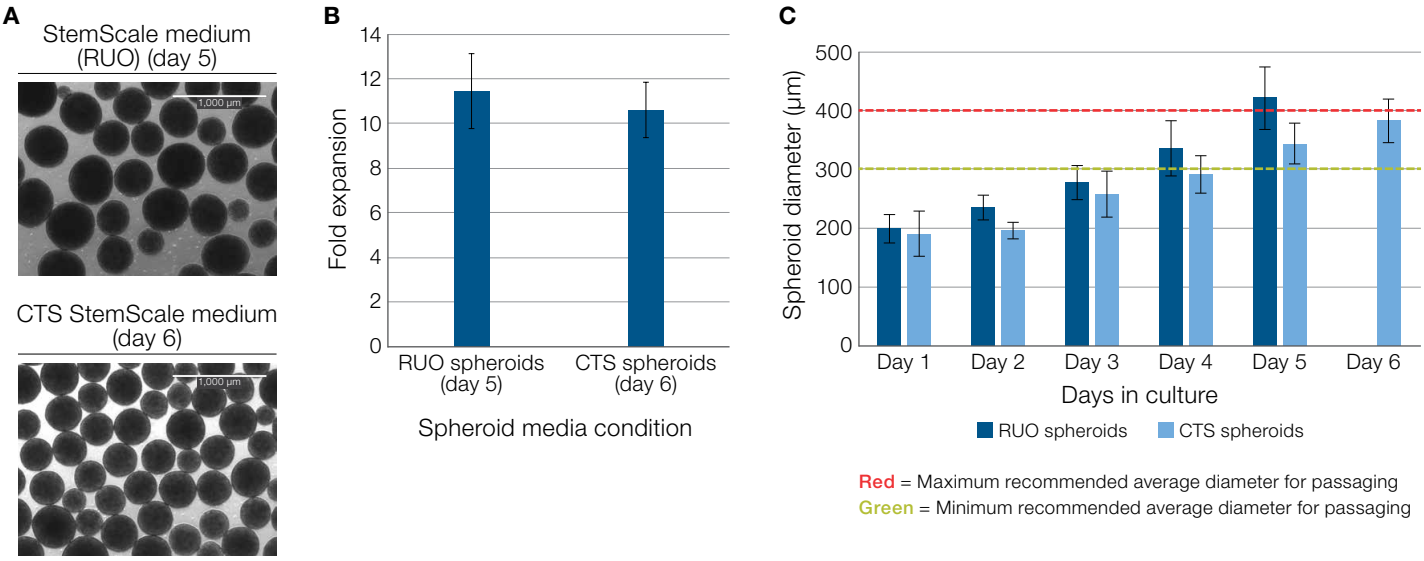


Figure 1. CTS StemScale medium provides similar performance to RUO StemScale medium. As indicated in Table 1, spheroids grown in CTS StemScale medium will require an additional day of growth as compared to spheroids grown in RUO StemScale medium to achieve similar cell yields. **(A)** Spheroid morphology on passage day. Spheroids grown in RUO StemScale medium will typically reach an average of 400 µm in diameter in 5 days, while spheroids grown in CTS StemScale medium will require an additional day to reach a similar diameter. **(B)** Cumulative cell expansion on passage day. By harvesting spheroids grown in RUO StemScale medium on day 5 and spheroids grown in CTS StemScale medium on day 6, it is possible to achieve similar total cell yields (reported as fold expansion). **(C)** Spheroid diameter comparison. The spheroid diameter of the spheroids grown in RUO StemScale medium and the spheroids grown in CTS StemScale medium are similar on the respective days of harvest, with both close to the upper recommendation of 400 µm in diameter.

Table 1. Key protocol differences between RUO StemScale and CTS StemScale media.

	RUO StemScale medium	CTS StemScale medium
Feeding strategy	Every other day	Daily
Days of growth	4–5 days*	5–6 days*
Seeding density	150,000 cells/mL	200,000 cells/mL
Dissociation reagent	Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent**	Gibco™ CTS™ TrypLE™ Select Enzyme (diluted†)
Inclusion of DNase I	DNase I not required	DNase I should be added

* Estimated time to achieve an average spheroid diameter between 300–400 µm.

** Contains an animal-origin component.

† Gibco™ CTS™ DPBS(-/-) can be used to dilute CTS TrypLE Select Enzyme to a lower concentration.

"StemScale medium is indeed robust and allows us to replicate results despite the cell line that we used. Its robustness allows us also to obtain very similar results again between different passages or even between different cell lines."

– Cláudia Miranda, Instituto Superior Técnico, University of Lisbon

Exceptional expansion capability

Advantages of StemScale PSC Suspension media:

- Achieve 5- to 10-fold expansion per passage
- Experience up to three times the expansion compared to other media
- Maintain optimal spheroid size and viability over culture duration

RUO StemScale medium

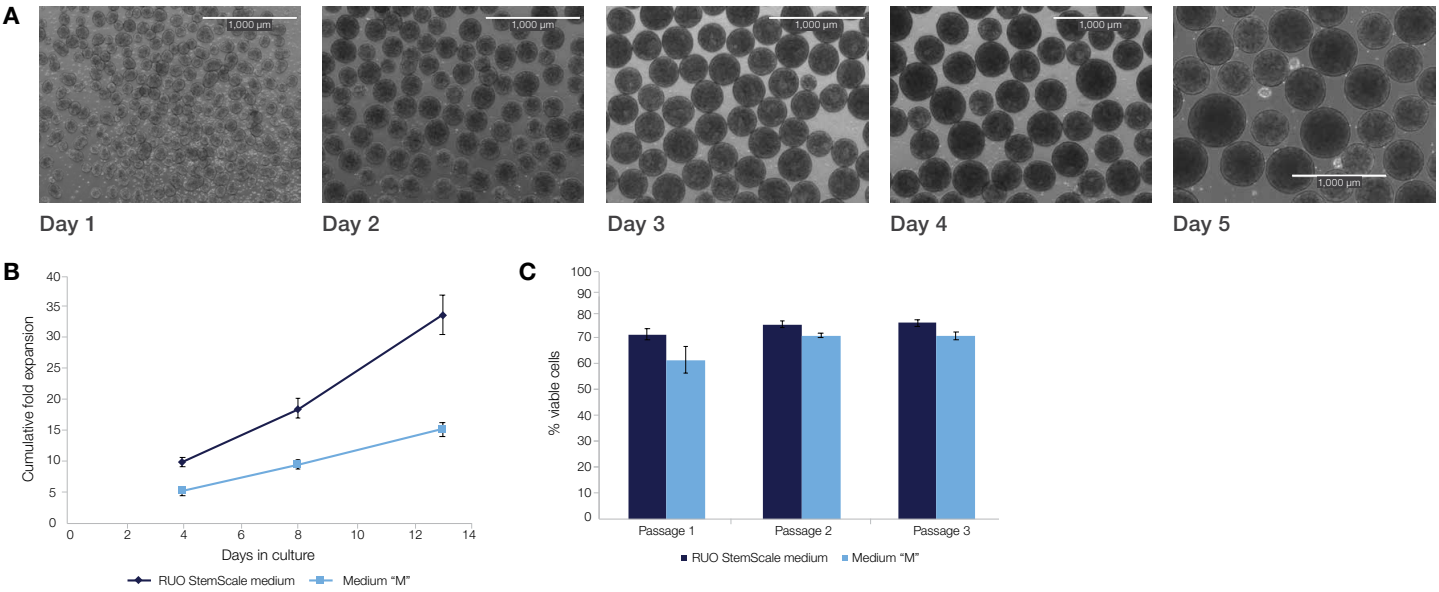


Figure 2. RUO StemScale medium delivers superior expansion capability. Multiple cell lines were cultured in Thermo Scientific™ Nunc™ non-treated 6-well plates in RUO StemScale medium. **(A)** Morphology and growth of PSC spheroids Days 1–5 after initiating cultures in RUO StemScale medium. **(B)** Fold expansion over 3 passages of PSCs cultured in RUO StemScale medium or in another supplier's medium "M". **(C)** Viability over 3 passages of PSCs cultured in RUO StemScale medium or in medium "M".

CTS StemScale medium

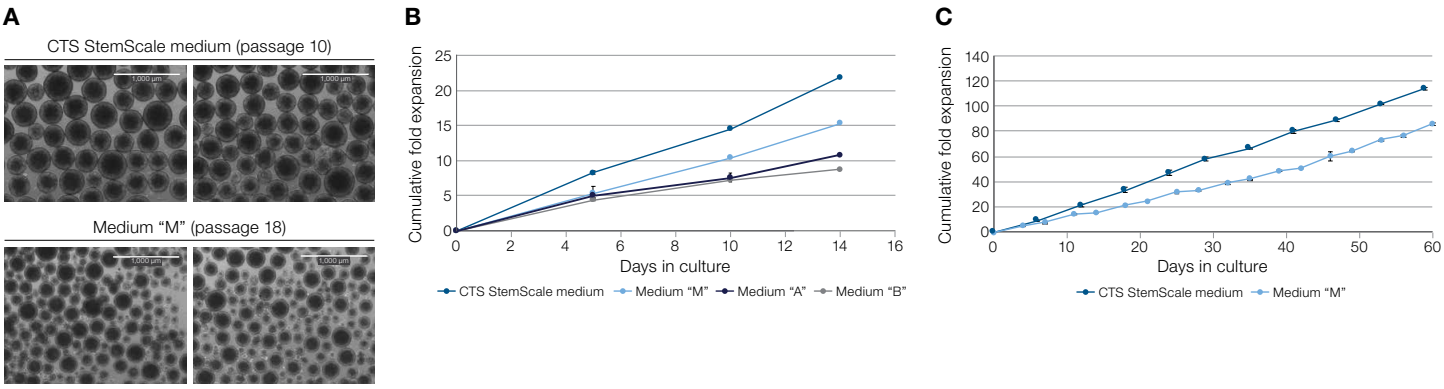


Figure 3. CTS StemScale medium delivers higher expansion potential than other PCS suspension media. (A) Representative spheroid morpholgy. CTS StemScale medium was compared against a commercially available suspension culture medium "M". Spheroids grown in CTS StemScale medium were larger and exhibited a more consistent spheroid morphology compared to the spheroids grown in medium "M". **(B)** Spheroid expansion in various PSC suspension culture media. Multiple PSC suspension media were selected for an initial 3-passage study against CTS StemScale medium. **(C)** Long-term competitive analysis: CTS StemScale medium vs. medium "M". After the initial 3-passage study, medium "M" was selected for a long-term study against CTS StemScale medium, with each medium using its respective protocol and passaging recommendation. In both studies **(B)** and **(C)**, CTS StemScale medium outperformed medium "M" and other media (medium "A" and medium "B") by exhibiting at least 25% greater expansion potential. CTS StemScale medium also required fewer passages compared to medium "M", reducing hands-on time and allowing for a more flexible culture schedule.

Simplified workflow

Other methods to culture pluripotent stem cells in suspension, such as the use of microcarriers or other self-aggregating methods, can be inconvenient during passaging, because researchers must either handle cells using cell strainers or dissociate the cells from the microcarriers. Additionally, the batch-feed method of feeding cells can lead to an over-accumulation of waste and inconsistent media volumes, which contribute to inconsistency of spheroid size. StemScale PSC Suspension Medium (RUO) utilizes a simplified approach to feeding cells, relying on gravity sedimentation and a 50% medium replacement strategy that is flexible and can be weekend-free.

Passaging of cultures does not require the use of cell strainers, which can lead to cell loss.

- **A flexible feeding schedule**—skip feeding days and passage as early as day 3
- **A 50% medium replacement feeding method**—prevents waste accumulation and contributes to consistent spheroid size
- **A straightforward passaging protocol**—no need for cell strainers, which makes it amenable to scale-up and does not require the use of microcarriers

RUO StemScale medium

Figure 4. Simplified workflow for adapting adherent cultures to suspension cultures using StemScale PSC Suspension Medium (RUO). After initiation of cultures in RUO StemScale medium, cells are fed periodically using a 50% medium exchange—every day or **(A)** every other day. **(B)** PSC cultured in plates or flasks are sedimented via gravity and 50% medium is aspirated and replaced with fresh, prewarmed medium, reducing metabolic waste in the medium. The plate or flask is mixed before placing back on the shake platform in the incubator. **(B, top)** Cells cultured in 6-well plates are first swirled to bring cells toward the center of the well, then the plates are tilted at a 45° angle. Spheroids can be imaged under the microscope directly in a 6-well plate. **(B, bottom)** A similar protocol is followed with cells grown in shaker flasks. Spheroids can be viewed under a microscope by taking a sample from the flask and transferring it to a 6-well plate. For detailed instructions on transitioning to suspension cultures using RUO StemScale medium, refer to the instructions in the user guide.

CTS StemScale PSC Suspension Medium also utilizes a simplified approach to feeding and passaging cells with a few key differences (Table 1).

- **A daily feeding schedule**—daily feed required; option to skip one day on the weekend
- **A 50% medium replacement feeding method**—prevents waste accumulation and contributes to consistent spheroid size
- **A straightforward passaging protocol**—no need for cell strainers, amenable to scale-up

CTS StemScale medium

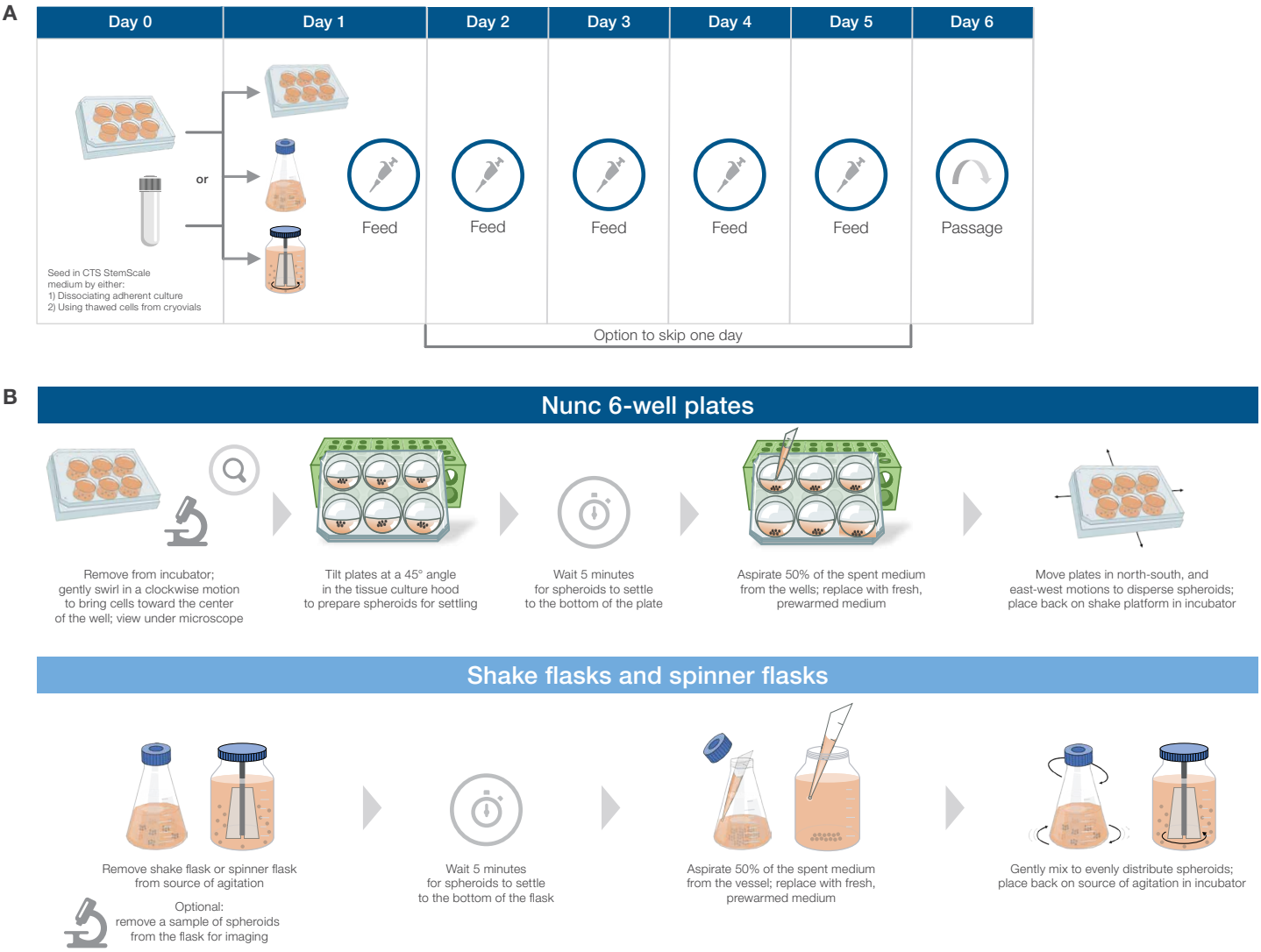


Figure 5. Simplified workflow for initiation of small-scale suspension cultures that can be scaled up. (A) Cultures in CTS StemScale medium are initiated by seeding single cells in the presence of ROCK inhibitor and DNase I. **(B)** Cultures in various vessel types are fed daily by replacing 50% of the medium. Once spheroids reach approximately 300–400 µm in diameter, they can be passaged via enzymatic dissociation. The resulting single cell suspension can then be scaled up into larger vessels. For detailed instructions, refer to the user guide.

Scalable across formats

For applications that require large volumes of cells, it is important to transition from smaller formats to larger vessels without sacrificing performance. Additionally, the workflow must allow for the flexibility to scale up. StemScale PSC Suspension media allow for transition from 6-well plates to flasks and bioreactors, while maintaining expansion capability. The StemScale PCS Suspension media protocols do not not require the use of cell strainers during passaging, which can be cumbersome and lead to cell loss when scaling across formats.

RUO StemScale medium

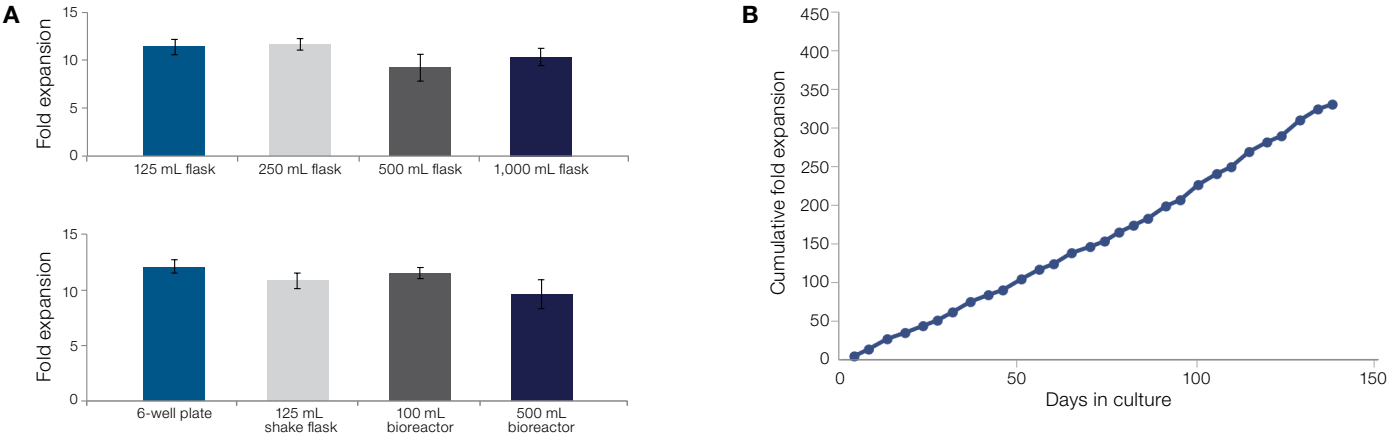


Figure 6. StemScale PSC Suspension Medium (RUO) is compatible across vessel types and sizes. (A) Cell expansion in Nalgene flasks (top) and other cell culture vessels (bottom). RUO StemScale medium achieved 9–12x expansion across multiple vessel types, including flasks and bioreactors. Maintenance of PSC expansion capability was achieved starting in 125 mL Thermo Scientific™ Nalgene™ flasks up to 1,000 mL, and then from Nunc 6-well plates up to a 500 mL bioreactor. **(B)** Long-term culture of human episomal iPSCs in 6-well plates. Over 30 passages and 20 weeks in culture, RUO StemScale medium delivers >10x expansion per passage.

CTS StemScale medium

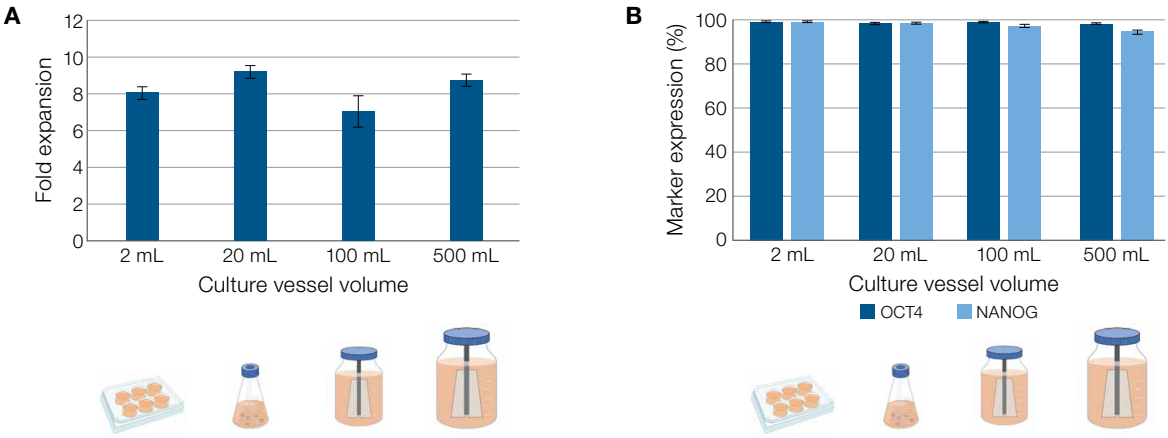


Figure 7. CTS StemScale medium supports efficient growth across multiple culture vessel sizes. (A) Cumulative growth after 6 days. The recommended seeding density of 200,000 cells/mL was used for cultures in CTS StemScale medium in all sizes of suspension culture vessels. Regardless of scale, cultures in CTS StemScale medium achieve a consistent high-fold expansion. **(B)** Spheroid pluripotency on passage day. Spheroids grown in CTS StemScale medium remain pluripotent in all different sizes of suspension culture vessels, as assessed through flow cytometric analysis of OCT4 and NANOG markers.

"StemScale PSC Suspension Medium improves upon the efficiency of stem cell aggregate formation."

– Sebastian Rieck, Process Development, ViaCyte Inc.

Consistency across cell lines and maintenance of high-quality cells

StemScale PSC Suspension media promote consistent spheroid formation and expansion capability across multiple PSC lines and maintain pluripotency across multiple passages.

Common challenges of PSC suspension culture are the inconsistent growth of cell lines adapted to suspension culture and the inability of some cell lines to reliably form spheroids when transitioning from adherent to suspension culture. StemScale PSC Suspension media are designed to improve the nucleation of single cells into spheroids and maximize cell growth across lines.

RUO StemScale medium

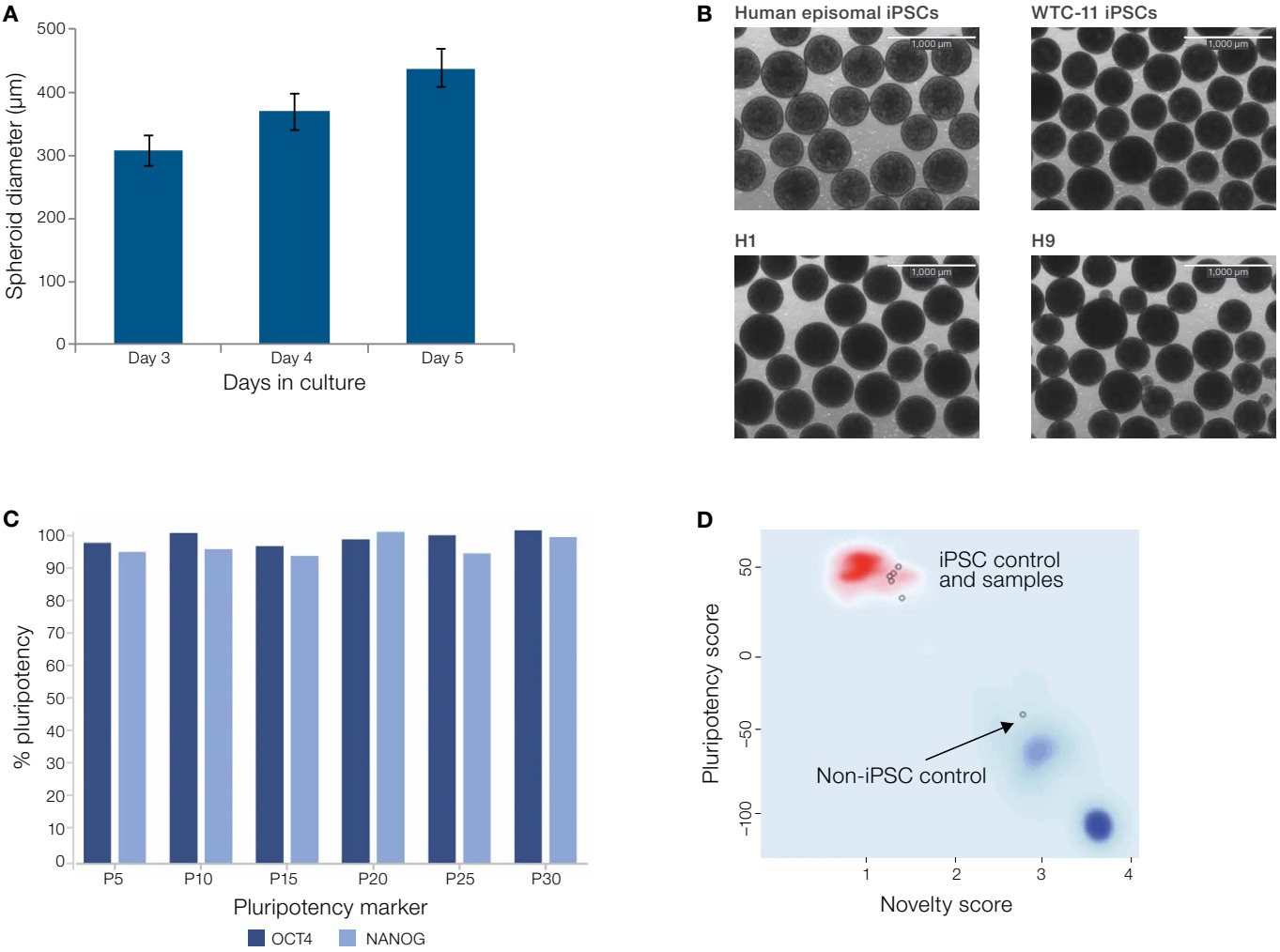


Figure 8. Consistent spheroid growth and maintenance of pluripotency. (A and B) Human ESC and iPSC lines were expanded in complete RUO StemScale medium. Consistent spheroid size and growth were observed across multiple cell lines after 5 days in culture. **(A)** Data were generated from an iPSC line. **(B)** Data were generated from iPSC and ESC lines. **(C)** Pluripotency assessment of OCT4 and NANOG markers. Gibco™ episomal iPSCs maintained in RUO StemScale medium for 30 consecutive passages exhibit >90% expression of pluripotent markers (OCT4 and NANOG) and normal karyotype (data not shown). **(D)** Pluripotency assessment with the PluriTest™ characterization assay. The PluriTest assay showed that PSCs maintained pluripotency across 30 passages. Additionally, these cells were found to maintain a normal karyotype, assessed using the Applied Biosystems™ KaryoStat™ Assay (data not shown).

CTS StemScale medium

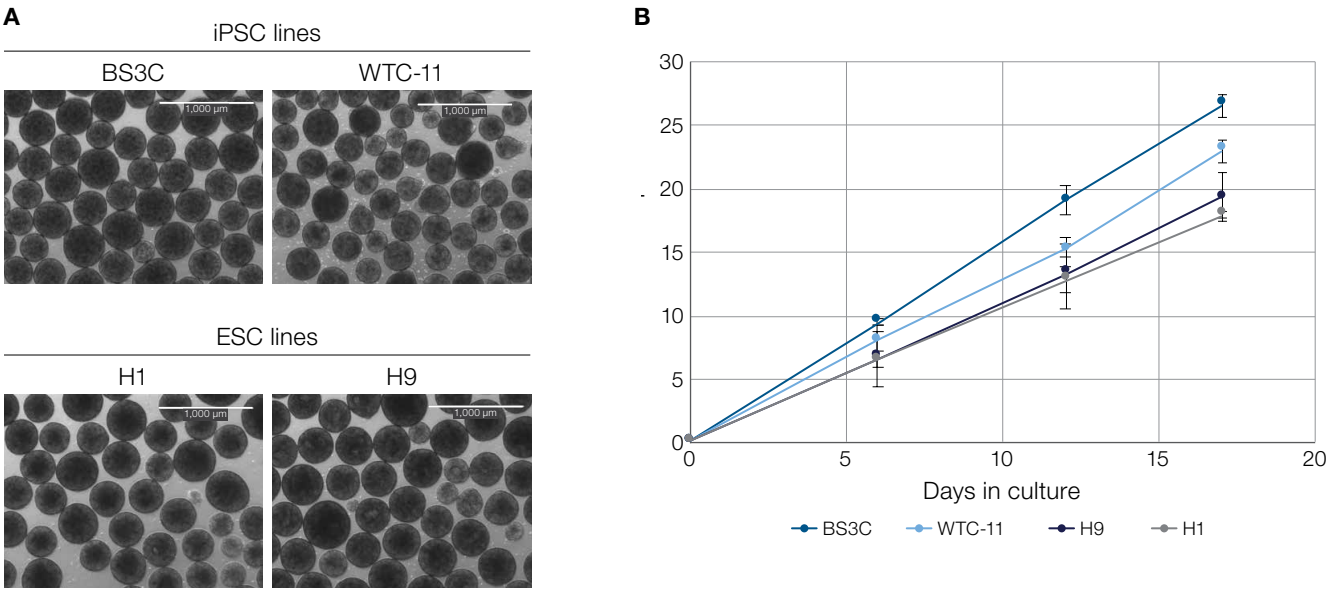


Figure 9. CTS StemScale medium supports the expansion of multiple cell lines. (A) Representative spheroid morphology at day 6**.** Although spheroid nucleation efficiency may vary between different cell lines, CTS StemScale medium enables multiple types of cell lines to nucleate and expand as spheroids. The images shown here are representative of spheroids after 6 days of growth in CTS StemScale medium. **(B)** Comparison of hPSC growth in CTS StemScale medium. Cell lines tested exhibited a range of 5x–10x expansion potential per passage. These results indicate that CTS StemScale medium supports the expansion capability of different PSC lines (BS3C, WTC-11, H9, and H1), with a typical cell line showing an average expansion potential per passage of 1 x 10^6–2 x 10^6 cells/mL. Since expansion is cell line dependent, it is important to become accustomed with how a particular line behaves when grown in CTS StemScale medium.

Differentiate PSC spheroids expanded in StemScale medium

The ability to create differentiated cells directly from PSC spheroids has been shown to save time and increase cell numbers compared to adherent methods.

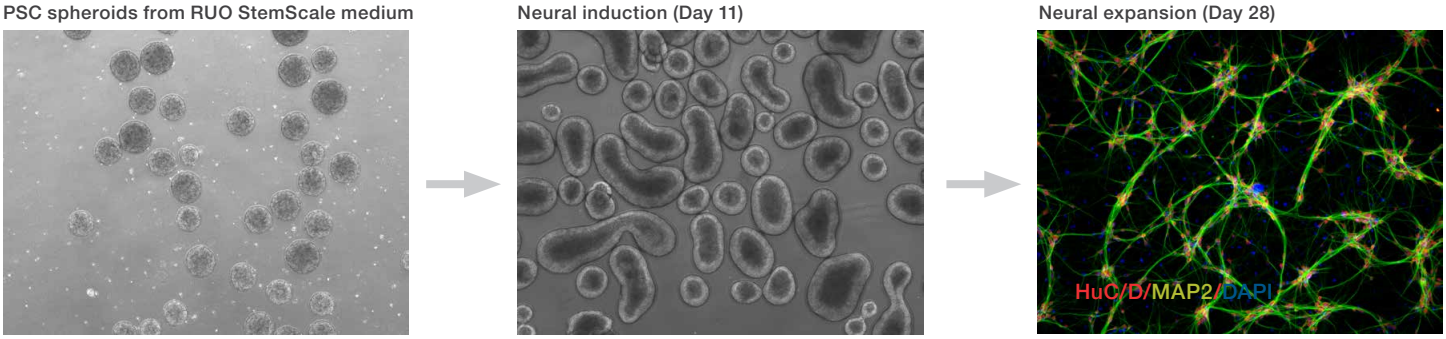


Figure 10. Differentiation can be initiated directly from PSC spheroids. Following expansion in StemScale PSC Suspension Medium (RUO), PSC spheroids transferred to Gibco™ PSC Neural Induction Medium were differentiated into neurons with high expression of phenotypic markers (HuCD and MAP2). The same number of neural stem cells can be achieved in just 11 days as compared to 27 days in adherent culture (data not shown). By day 32, the same number of mature neurons are achieved as at day 48 in adherent culture.

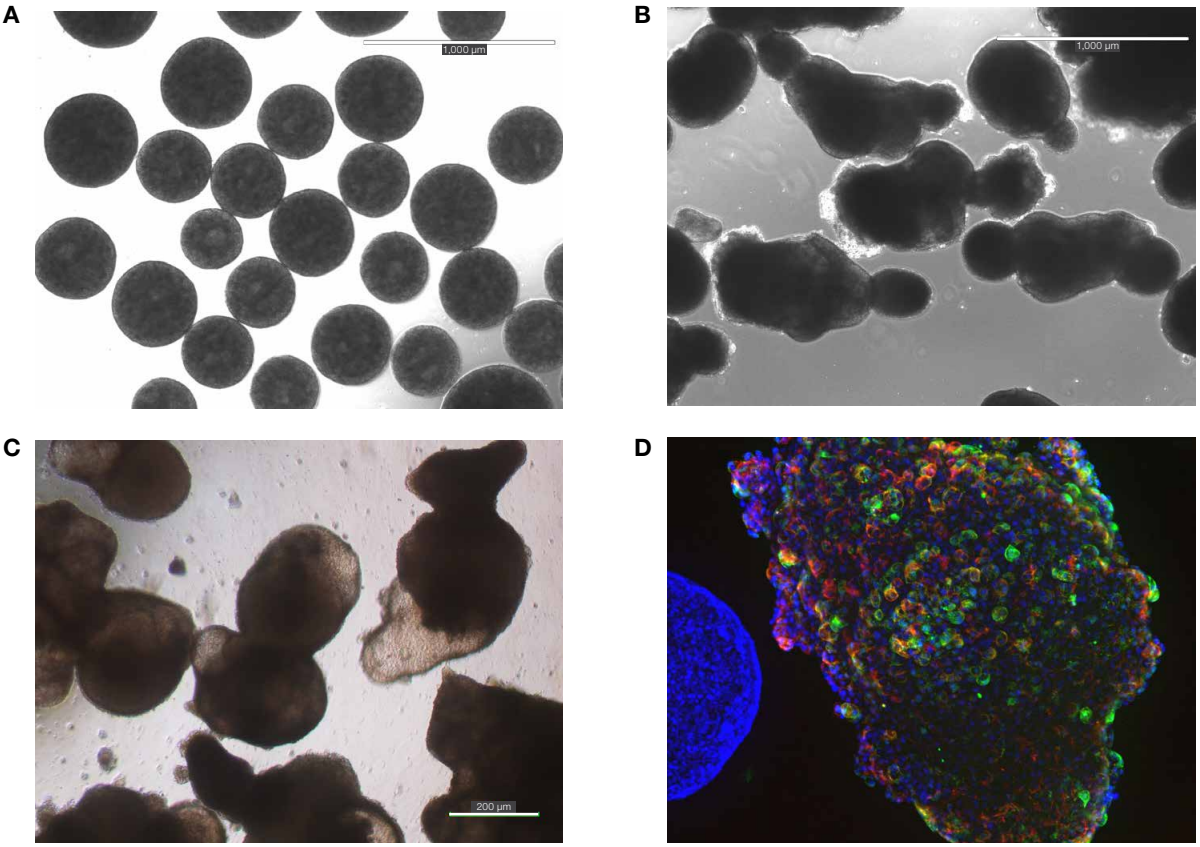


Figure 11. Cardiomyocytes are successfully differentiated in suspension from spheroids grown in StemScale PSC Suspension Medium (RUO). (A) Undifferentiated hPSC spheroids growing in RUO StemScale medium prior to cardiac induction. **(B)** Spheroids in rotation culture 12 days after start of cardiac induction. **(C)** Cardiac organoids exhibiting spontaneous contractions after two weeks of differentiation. **(D)** Whole-mount organoid staining for the cardiac markers alpha sarcomeric actinin (green) and cardiac troponin T (red). Nuclei are counterstained with DAPI (blue) stain.

Frequently asked questions (FAQs)

Q: Do StemScale PSC Suspension media maintain the pluripotency of human pluripotent stem cells (hPSCs) in suspension culture?

A: Yes, RUO StemScale medium and CTS StemScale medium maintain the pluripotency of hPSCs grown as spheroids in suspension culture over 30 passages as assessed by both flow cytometric analysis and the PluriTest assay.

Q: Do StemScale PSC Suspension media maintain normal karyotype of hPSCs in suspension culture?

A: Yes, RUO StemScale medium and CTS StemScale medium maintain normal karyotype of hPSCs grown as spheroids in suspension culture over 30 passages as assessed by the KaryoStat assay.

Q: Are StemScale PSC Suspension media able to maintain high viability of hPSCs in suspension culture?

A: Yes, trypan blue staining of single cells obtained from dissociated spheroids indicates that the viable cell count of suspension cultures remains high (>90%).

Q: Do StemScale PSC Suspension media maintain trilineage differentiation potential of hPSCs in suspension culture?

A: Yes, RUO StemScale medium and CTS StemScale medium maintain trilineage differentiation potential of hPSCs as assessed by analysis with the Applied Biosystems™ TaqMan™ hPSC Scorecard™ Panel.

Q: Are StemScale PSC Suspension media compatible with multiple different cell lines?

A: Yes, we have evaluated multiple different iPSC and ESC cell lines, all of which were demonstrated to be compatible with RUO StemScale medium and CTS StemScale medium.

Q: What vessels are suitable for my suspension cultures grown with StemScale PSC Suspension media?

A: A variety of culture vessels, including non–tissue culture treated well plates, plain-bottom shaker flasks, and bioreactors, are suitable for suspension cultures grown with RUO StemScale medium and CTS StemScale medium.

Q: Do complete StemScale PSC Suspension media require the use of microcarriers to form spheroids?

A: No, RUO StemScale medium and CTS StemScale medium promote the formation of spheroids through self-aggregation.

Q: What is the recommended passaging schedule when using StemScale PSC Suspension media?

A: The RUO StemScale medium protocol recommends passaging every 4–5 days, depending on spheroid size. The CTS StemScale medium protocol recommends passaging every 5–6 days, depending on spheroid size. It is recommended to passage suspension cultures when the average spheroid diameter is between 300–400 µm.

Passaging cultures earlier is also an option but may result in a lower cell yield. Early passaging is an option to avoid passaging on a weekend.

Q: Do I need to feed my cultures daily with StemScale PSC Suspension media?

A: The RUO StemScale medium feeding schedule allows for every-other-day feeding.

While suspension cultures can still be fed daily to maximize cell health, RUO StemScale medium allows users the flexibility to feed suspension cultures every other day without sacrificing performance.

However, the CTS StemScale medium feeding schedule recommends daily feeding to maximize cell health. It is possible to skip one day on the weekend if desired, but otherwise CTS StemScale medium cultures should always be fed daily.

Q: Should I be concerned about waste by-products accumulating in my suspension cultures using the feeding method of StemScale PSC Suspension media?

A: No, the feeding method utilized with RUO StemScale medium and CTS StemScale medium involves replacing 50% of the spent medium with fresh medium. This feeding method prevents spheroids from being cultured in medium that is accumulating significant quantities of waste by-products.

Q: Are there advantages to the feeding method of StemScale PSC Suspension media as compared to feeding methods for other media using suspension cultures?

A: The feeding method utilized with RUO StemScale medium and CTS StemScale medium 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 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.

Q: How do the passaging protocols for the StemScale PSC Suspension media compare to protocols for other media using suspension cultures?

A: The RUO StemScale medium protocol recommends passaging every 4–5 days, depending on spheroid size. The CTS StemScale medium protocol recommends passaging every 5–6 days, depending on spheroid size. We recommend passaging suspension cultures when the average spheroid diameter is between 300–400 µm, which occurs after approximately 4–5 days of growth for the RUO StemScale medium and after 5–6 days of growth for the CTS StemScale medium. Passaging earlier is also an option, although the final cell yield will be lower than what is typically obtained.

Passaging protocols for other suspension culture media may offer less flexibility, depending on if 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 PCS Suspension media passaging protocols do not require the use of microcarriers or cell strainers, while other PSC suspension culture medium protocols may require such tools.

Ordering information

Product	Unit size	Cat. No.
StemScale PSC Suspension Medium	1 L	A4965001
CTS StemScale PSC Suspension Medium	1 L	A5869601
StemPro Accutase Cell Dissociation Reagent	100 mL	A11105
CTS TrypLE Select Enzyme	100 mL	A1285901
DNase I, RNase-free	1,000 units	EN0521
CultureCEPT Supplement	0.1 mL	A56800
	0.5 mL	A56799
Human Episomal iPSC Line, or other human iPSCs or ESCs	1 x 10^6 cells/vial	A18945
Antibiotic-Antimycotic (100X)	100 mL	15240062
Trypan Blue Solution, 0.4%	100 mL	15250061
DPBS, no calcium, no magnesium	500 mL	14190250
CTS DPBS, no calcium, no magnesium	1 L	A1285601
	2 L	A1285602
PSC Neural Induction Medium	500 mL	A1647801
Recommended plates, accessories, and other equipment		
Nunc Non-Treated Multidishes	6-well	150239
	24-well	144530
Nunclon Sphera plates	Case of 7	174932
Nalgene Single-Use PETG Erlenmeyer Flasks with Plain Bottom, Sterile	125 mL	4115-0125
	250 mL	4115-0250
	500 mL	4115-0500
CO_2 Resistant Shakers	1 unit	88881101 (North America only)
		88881102
Rubber Mat Platform (for CO_2 Resistant Shakers)	1 mat platform	88881123
Countess 3 FL Automated Cell Counter	1 instrument	AMQAF2000
EVOS XL Core Imaging System	1 instrument	AMEX1000

Learn more at **thermofisher.com/stemscale**

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