

CET COCKTAIL

Small molecule kit designed to minimize cellular stress and enhance stem cell survival

Human pluripotent stem cells (hPSCs) have extensive self-renewal capacity in culture, yet are highly sensitive to their in vitro environment, posing a challenge to their long-term culture and downstream differentiation. A targeted high-throughput analysis has found that the combination of **Chroman 1**, **Emricasan**, and **Trans-ISRIB** small molecules (**CET**), used together with a polyamine solution, can be safely used in research and regenerative medicine applications to improve the viability and expansion of hPSCs in culture, as well as improve functionality of derived cell types¹.

The CET Cocktail is comprised of the key small molecule components in the media supplement strategy known as CEPT (Chroman 1, Emricasan, Polyamine solution, and Trans-ISRIB). CEPT is a published, optimized combination of targeted small molecules and culture additives proven to enhance stem cell survival and address the key challenges in translational stem cell research. The small molecules in the CET Cocktail provide comprehensive cytoprotection to overcome cellular stressors inherent in stem cell workflows.

Features of the Captivate Bio CET Cocktail:

- Complete kit of key small molecules in published CEPT recipe
- Compatible with common hPSC culture media, substrates, and techniques
- Cost-effective solution for improved stem cell workflows



Reliable solution for improved stem cell workflows

Supplementation of small molecules in the CET Cocktail has been shown to directly and efficiently enhance pluripotent stem cell survival and promote clonal growth and expansion of genetically stable hPSCs¹. The published CEPT combination allows for safe, controlled, and efficient strategies for the generation, expansion, differentiation, and banking of stem cell-based therapeutic products. This small molecule cocktail is also key for optimized embryoid body (EB) and organoid formation, as well as superior cryopreservation of pluripotent and differentiated cells by protecting cells from multiple cellular stress mechanisms in a synergistic fashion¹.

For best results, the CET Cocktail is intended to be used according to published concentrations and workflow following the publication from Yu Chen et al. 2021 Nature Methods. This includes the addition of Polyamine Solution to generate the complete CEPT supplement prepared in cell culture media just before use.

Advantages of using the CET Cocktail:

- More effective than standard treatments
- Improved survival of dissociated cells in culture
- Higher yield of viable hPSC clones
- Validated to be safe for long-term hPSC culture

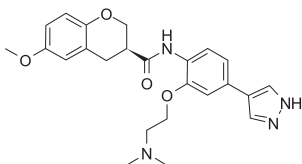


Applications

- Routine passaging of hPSCs in culture
- Single-cell cloning and gene editing
- iPSC reprogramming methods
- Embryoid body and organoid formation
- Cryopreservation and cell banking

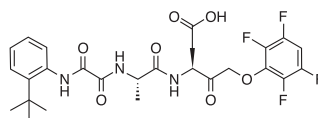
CET Cocktail Kit

The Captivate Bio CET Cocktail contains three key small molecules for enhancing stem cell survival. Each kit includes:



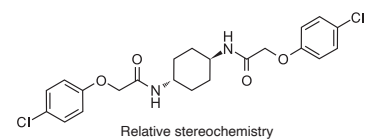
Chroman 1, 5mg

- Highly potent and selective ROCK inhibitor
- No demonstrated off-target effects at standard dose



Emricasan, 10 mg

- Potent and irreversible pan-caspase inhibitor
- Enhances cell survival through synergistic effects with Chroman 1



Trans-ISRIB, 10 mg

- Integrated stress response (ISR) inhibitor
- Key component in CEPT cocktail shown to enhance stem cell survival

Enhanced hPSC survival and viability

The combination of the CET Cocktail small molecules (Chroman 1 at 50 nM, Emricasan at 5 μ M, and Trans-ISRIB at 0.7 μ M) with Polyamine Solution (CEPT) promotes clonal growth and expansion of genetically stable hPSCs. Supplementation with CEPT provides chemical protection and overcomes inherent survival issues with single-cell dissociation, offering a new approach for safe and efficient hPSC culture.

The following figures are represented data published from Yu Chen, et al. (2021) A versatile polypharmacology platform promotes cytoprotection and viability of human pluripotent and differentiated cells. Nature Methods. May; 18(5): 528-514.

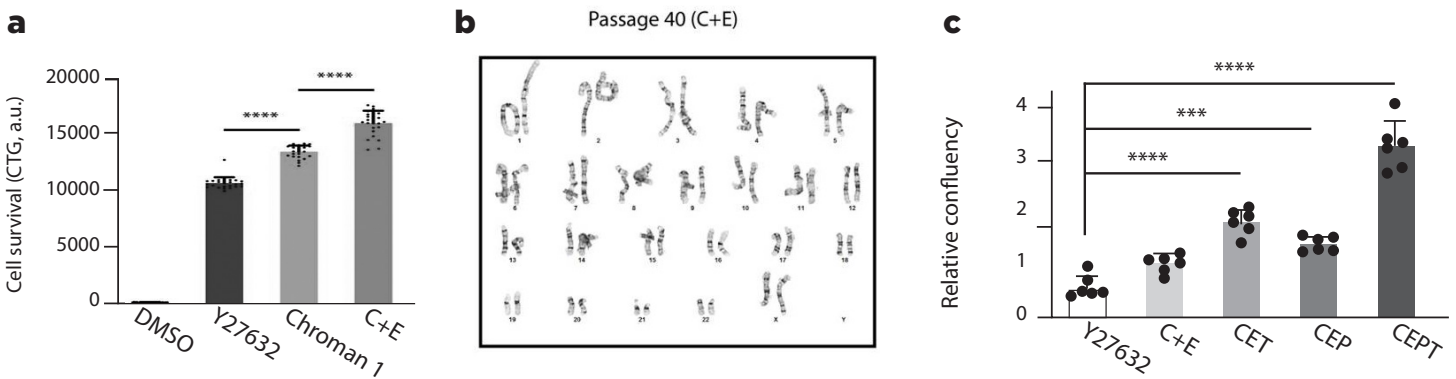


Figure 1. Small molecules enhance cell survival. When the C+E small molecule combination of Chroman 1 (50 nM) and Emricasan (5 μ M) was applied to hPSCs for the first 24 hours after single-cell dissociation, a significant increase in the number of viable cells was observed 24 hours post-seeding, as compared to treatment with Y27632 or Chroman 1 alone (a). Cells remained karyotypically normal when dissociated as single cells for 40 passages and treated with Chroman 1 and Emricasan during the first 24 hours of every passage (b).

Figure 2. Cell survival. hPSCs were seeded as single cells at an ultra-low density of just 25 cells/cm² and incubated with Y27632 and various combinations of the CEPT small molecule and polyamine components, demonstrating combinatorial improvements to cell survival (c).

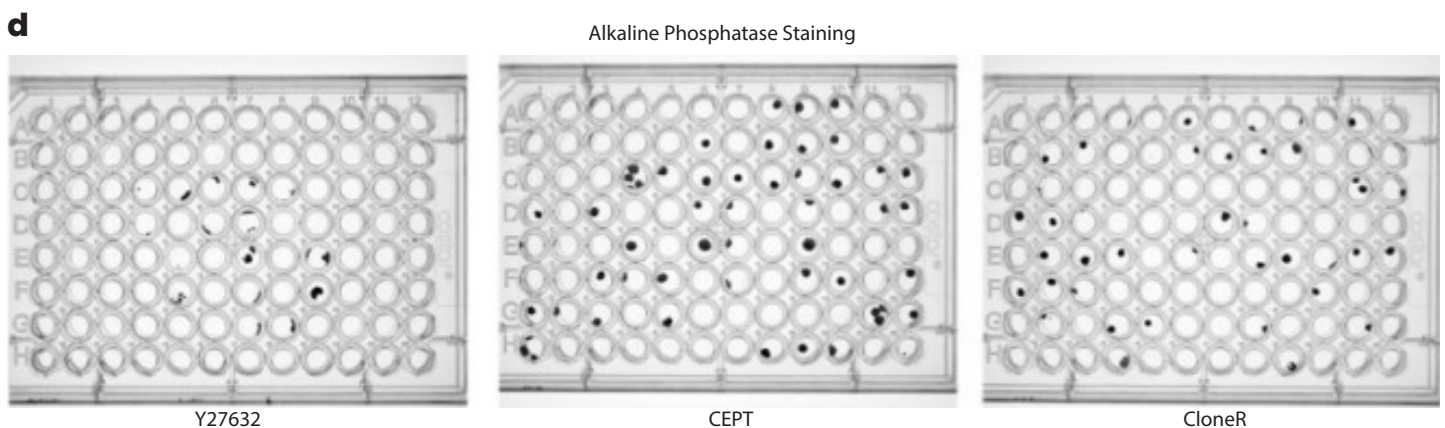



Figure 3. Improved clonal growth and expansion of hPSCs. An alkaline phosphatase staining assay of hPSCs cultured with Y27632, the complete CEPT combination, and commercially available CloneR™ supplement, clearly showed more alkaline phosphatase-positive clones in cultures exposed to CEPT (d).

CET Cocktail

REF CET01B

 -20° C

The CET Cocktail contains one vial each of the following small molecules:

Product	Size	Cat. No.
Chroman 1	5 mg	SML20A
Emricasan	10 mg	SML21B
Trans-ISRIB	10 mg	SML22B

Storage

Store the CET Cocktail kit tightly sealed as lyophilized powder or as separate aliquots of prepared concentrated stock solutions.

The final working solution of each compound should be made independently just before use in appropriate cell culture media.

Lyophilized - Store vials tightly sealed in their original container for up to 3 years at -80°C, or up to 2 years at -20°C.

In Solvent - Store individually at concentrated stock concentrations for up to 6 months at -80°C, or up to 1 month at -20°C. Avoid freeze/thaw cycles.

References

References are publications that support the biological activity of the product.

1. Yu Chen, et al. (2021) A Versatile Polypharmacology Platform Promotes Cytoprotection and Viability of Human Pluripotent and Differentiated Cells. *Nature Methods*. May; 18(5): 528-514
2. Carlos A Tristan et al. (2021) Robotic high-throughput biomanufacturing and functional differentiation of human pluripotent stem cells. *Stem Cell Reports*, Vol. 16, Issue 12, 3078-3092

Refer to individual small molecule Technical Data Sheets and SDS for additional compound storage, solubilization, and safety information.

Instructions for Use

The CET Cocktail is intended to be used according to published concentrations and workflow described in Yu Chen et al. 2021 *Nature Methods*, including the addition of Polyamine Solution. According to the publication, the CET Cocktail compounds are used at the following concentrations:

- **Chroman 1:** 50 nM
- **Emricasan:** 5 µM
- **Trans-ISRIB:** 0.7 µM

1. Prepare stock solutions for each compound.

Chroman 1

1. To prepare a 0.5 mM (10,000X) solution, reconstitute 5 mg lyophilized Chroman 1 compound in 22.91 mL of DMSO.
2. Vortex solution until the Chroman 1 is completely dissolved.
3. Aliquot the stock solution to smaller working volumes.

Emricasan

1. To prepare a 50 mM (10,000X) solution, reconstitute 10 mg lyophilized Emricasan compound in 0.3512 mL of DMSO.
2. Vortex solution until the Emricasan is completely dissolved.
3. Aliquot the stock solution to smaller working volumes.

Trans-ISRIB

1. To prepare a 7 mM (10,000X) solution, reconstitute 10 mg lyophilized Trans-ISRIB compound in 3.165 mL of DMSO.
2. Gently warm the solution between 45°C and 60°C, and vortex until the Trans-ISRIB is completely dissolved.
3. Aliquot the stock solution to smaller working volumes.

2. Prepare supplemented media aliquot.

1. Just prior to use, supplement an aliquot of complete hPSC culture media with the CET small molecules 1:10,000.
 - Add 0.1 µL of 10,000X stock solution of each small molecule per 1 mL of medium.
2. Add 1 µL of 1000X Polyamine Solution per 1 mL of medium.
3. Filter the prepared medium using a 0.2 µM pore size filter before use.

Ordering information

PRODUCT	COMPONENTS	SIZE	CAT. NO.
CET Cocktail	Chroman 1, 5 mg (SML20A)	1 Kit	CET01B
	Emricasan, 10 mg (SML21B)		
	Trans-ISRIB, 10 mg (SML22B)		

RELATED PRODUCTS	APPLICATION	SIZE	CAT. NO.
Y27632 (2HCl)	ROCK inhibitor; cell survival	10 mg	SML13B
DMEM/F12 Medium (with HEPES)	Stem cell culture	500 mL	DFHG01
DPBS (without Ca ⁺⁺ and Mg ⁺⁺)	Cell culture; wash solution	1000 mL	DPBS02

For bulk requests, custom small molecule sourcing, or a complete list of small molecules currently available from Captivate Bio, please visit our website at www.captivatebio.com, email orders@captivatebio.com, or contact our customer service team at (617) 607-4017.

Together, we make breakthroughs happen.

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Reference and data presented supports the the biological activity of a product. Always refer to the authors and full publication referenced for more information. The data provided herein may relate to products or workflows that have not been fully validated by Captivate Bio and is subject to change without notice.

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