

## Protocol for CEPT Cocktail Kit

### In Brief

CEPT Cocktail is a combination of small molecules, Chroman 1 (Cat. No. 7163), Emricasan (Cat. No. 7310), Polyamine Supplement x1000 (lyophilized) (Cat. No. 7739), and trans-ISRIB (Cat. No. 5284) available from Tocris, which improves the viability of pluripotent stem cells (PSCs). This combination has been shown to be effective in cryopreservation, routine long-term passaging, as well as embryoid body and organoid formation.

### CEPT Cocktail Kit

Product	Catalog Number	Unit Size	Stock Solution Concentration	Volume for Reconstitution
<b>Chroman 1</b>	7163	1 vial	10,000x	500 µL DMSO for a 0.5 mM solution
<b>Emricasan</b>	7310	1 vial	10,000x	500 µL DMSO for a 50 mM solution
<b>trans-ISRIB</b>	5284	1 vial	10,000x	500 µL DMSO for a 7 mM solution
<b>Polyamine Supplement x1000 (lyophilized)</b>	7739	1 vial	1000x	5 mL ddH <sub>2</sub> O
<b>DMSO, sterile filtered</b>	3176	5 mL		

### Reconstitution of stock solutions

1. Prepare stock solutions (10,000x) for Chroman 1 (0.5 mM), Emricasan (50 mM) and trans-ISRIB (7 mM) by adding 500 µL of DMSO, sterile filtered, to each vial. Mix thoroughly or gently warm the solutions in a 45-60°C water bath to ensure complete reconstitution. Add 100 µL of resuspended 10,000x stock solution per 1 L of media.
2. Prepare a 1000x Polyamine Supplement stock solution by reconstituting vial contents in 5 mL of ddH<sub>2</sub>O and filter sterilize before use. Add 1 mL of sterile resuspended polyamines supplement (1000x stock) per 1 L of media.
3. Filter the prepared medium before use.

### Storage and Handling

Store the stock solutions, once prepared, at -80°C and use them within 6 months, or at -20°C within 3 months.