Enriching Human Midbrain-Hindbrain Organizer Cells from Stem Cell Cultures

Offen et al. (2023). Enrichment of FGF8-expressing cells from neurally induced human pluripotent stem cell cultures; **Stem Cell Reports**, 18(11), 2240-2253. DOI: 10.1016/j.stemcr.2023.09.011.

iPSC; embryoid bodies; FACS standardization	
Index	SC9
Standardization	X
Counting	X
Viability	
Volume	

The Challenge:

Precisely identifying and isolating rare, functional morphogen-secreting cells—specifically those mimicking the midbrain-hindbrain organizer—from highly heterogeneous human pluripotent stem cell (hPSC) cultures.

CASY's Contribution:

The CASY cell counter was used to provide high-precision automated cell counts of dissociated embryoid bodies. This allowed researchers to accurately adjust cell concentrations to 1x10^6 cells per 100 µL in FACS buffer, ensuring optimal and standardized conditions for the subsequent fluorescence-activated cell sorting of organizer-like populations.

Key Benefits to Researchers:

- **FACS Standardization**: Provided the exact cell densities necessary to set reproducible sorting gates for rare SEF^{high}/18R5^{low} populations.
- **Quantitative Accuracy**: Enabled precise normalization of cell numbers for downstream functional assays, including the transplantation of 2,500 cells per collagen particle into chick embryos.
- **Protocol Reproducibility**: Ensured that the technical starting point for each enrichment experiment was identical, reducing variability in the isolation of transcriptionally distinct cell clusters.

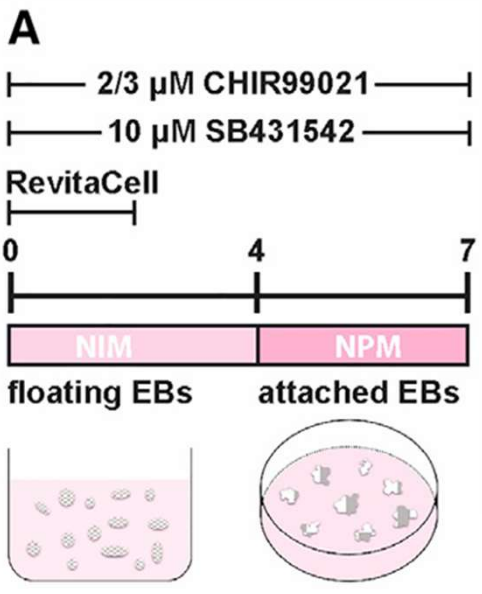

Figure 2A: (Cell culture protocol and flow cytometry-based sorting scheme). This figure outlines the transition from attached embryoid bodies to a single-cell suspension on day 7. CASY was utilized at this specific junction to quantify the cells before they were sorted into the P4 (SEF^{high}/18R5^{low}) population identified as the functional organizer.