Growth Protocol for ES-EM5Sox17huCD25

Differentiation protocol from Shin-Ichi Nishikawa's lab: For mesoderm/endoderm differentiation of ES-GscgfpSox17huCD25 ESCs, 2–3 3 105 cells were seeded onto type IV collagen-coated 10-cm dishes in SF-O3 medium (Sanko Junyaku) supplemented with 0.1%–0.3% bovine serum albumin, 50 mM b-mercaptoethanol, and 10 ng/mL activin A (R&D Systems, 338-AC/CF). After 6 d of culture, cells were labeled with BrdU for 2 h, immediately stained on ice with PE-conjugated anti-CD25 antibody (BD Pharmingen, 557138) to avoid cell-cycle progression, and Gsc+Sox17–mesoderm and Gsc+Sox17+ endoderm cells were collected by fluorescence activated cell sorting (FACS) as described (Yasunaga et al. 2005). Sorted cells were immediately fixed in 75% ethanol and further separated into early and late S-phase fractions by flow cytometry for replication profiling.

References

Yasunaga M, Tada S, Torikai-Nishikawa S, Nakano Y, Okada M, Jakt LM, Nishikawa S, Chiba T, Era T, Nishikawa S. Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. Nat Biotechnol. 2005 Dec;23(12):1542-50. Epub 2005 Nov 27.