

Naïve stem cell culture

t2iLGöY Media Formulation:

Cells are propagated in modified N2B27 medium supplemented with PD0325901 (1 μ M, prepared in house), CHIR99021 (1 μ M, prepared in house), Gö6983 (2.5 μ M, Sigma-Aldrich), Rho-associated kinase inhibitor (ROCKi, Y-27632)(10 μ M, Calbiochem), human LIF (10 ng/ml, prepared in house) and ascorbic acid (250 μ M, Sigma). This medium is referred to as t2iLGöY.

Modified N2B27 medium (1L) comprised 490 mL DMEM/F12 (LifeTechnologies), 490 mL Neurobasal (Life Technologies), 10 mL B27 (Life Technologies), 5mL N2 (prepared in house), 10 μ g/mL insulin (Sigma), 2 mM L-glutamine (Life Technologies) and 0.1 mM 2-mercaptoethanol (Sigma). N2 contains 100 μ g/ml apo-transferrin eBioscience, ABC2553), 3 μ M sodium selenite (Sigma), 1.6 mg/mL putrescine (Sigma) and 2 μ g/mL progesterone (Sigma) in DMEM/F12 (Life technology).

Passaging & Freezing:

Naïve cells are cultured on a feeder layer of mitotically-inactivated (irradiated) murine embryonic fibroblasts (MEFs). Naïve stem cells are passaged either manually with Accutase (Life Technologies) dissociation or as a pool using TrypLE™ Express (Life technology, 12605). Cells are cultured in 5% oxygen, 7% carbon dioxide in a humidified incubator at 37°C. Cells are frozen in 50% t2iLGöY medium supplemented with 40% serum and 10% DMSO.