

Differentiation Protocol for: Panc_CyT49

CyT49 hESCs were differentiated in a four-step sequential process to

Stage 1:

Definitive Endoderm

Stage 2:

primitive gut tube (HNF1B+, HNF4A+);

Stage 3:

posterior foregut (PDX1+);

Stage 4:

pancreatic endoderm (NKX6-1+, PTF1A+)(~16-47 %)

endocrine cells (NGN3+, NKX2-2+, PAX4+, PAX6+)(~40-65%)

Stage 1 (days 0-1): cells were maintained in RPMI 1640 (Mediatech), 0.2% FBS (HyClone), 1x GlutaMAX-1 (Life Technologies), 1% v/v penicillin/streptomycin, 1:5000 Insulin-Transferrin-Selenium (ITS) (Life Technologies), 100 ng/mL recombinant human Activin A (R&D Systems), 50 ng/mL recombinant mouse Wnt3A (R&D Systems).

Stage 2 (days 1-4): addition of 25 ng/mL recombinant human KGF (R&D Systems) and 2.5 mM TGF- β RI Kinase inhibitor IV (EMD Bioscience).

Stage 3 (days 5-7) cells were maintained in DMEM HI Glucose (HyClone) supplemented with 0.5x B-27 Supplement (Life Technologies), 1x GlutaMAX-1 and 1% v/v penicillin/streptomycin, 0.25 mM KAAD-Cyclopamine (Toronto Research Chemicals), 3 nM TTNPB (Sigma-Aldrich) and 50 ng/mL recombinant human Noggin (R&D Systems).

Stage 4 (days 8-12): supplemented with 50 ng/mL recombinant human KGF (R&D Systems) and 50 ng/mL recombinant human EGF (R&D Systems).

Reference:

Schulz et al. 2012. PLoS One. 7: e37004