Differentiation Protocol for: Liver_CyT49

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

Stage 1: Definitive Endoderm
Stage 2: Specified Hepatocytes
Stage 3: Immature Hepatocytes
Stage 4: Hepatocytes

Using modifications to pancreatic differentiation (medium supplemented with 100 ng/mL BMP4 (R&D Systems) and 25 ng/mL FGF2 (Sigma) to stages 2-4 resulting in an APF+, Albumin+ and TAT+ cell population.

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