

Dalton Lab Differentiation Protocols for ENCODE Project

Smooth Muscle Differentiation:

3x10⁶ WA09 hESCs are seeded per 100mm Geltrex coated dish in CDM with 100ng/ml BMP4 and 25ng/ml Wnt3a for 4 days at 37°C/5%CO₂ with daily media changes. On day 4, the media is changed to CDM with 50ng/ml BMP4 and 25ng/ml Wnt3a for 18 days with media changes every other day.

Neural Progenitor Differentiation:

3x10⁶ WA09 hESCs are seeded per 100mm Geltrex coated dish in NP media: DM with 10ng/ml Heregulin β1, 100ng/ml Long R3-IGF1, 20uM SB431542, and 0.5uM LDN193189. Cells are cultured at 37°C/5%CO₂. Media is changed daily for 5 days.

Hepatic differentiation:

6x10⁶ WA09 hESCs are seeded per well of a 6-well suspension culture plate in CDM with 10nM Y27632. Cells are cultured on a rotating platform in the 37°C/5%CO₂ incubator at 95 RPM. After 1-2 days, DE differentiation begins by changing the media to DM with 100ng/ml Activin A, 8ng/ml bFGF, 25ng/ml Wnt3a, and 10nM Y27632 (day 0). Media is changed daily, with Wnt3a removed on day 1 and Y27632 removed on day 2.

On day 3, media is changed to Hepatic Media A (HM-A)

On day 5, media is changed to HM-B, with changes every 2 days

On day 9, media is changed to HM-C, with changes every 2 days

On day 15, media is changed to HM-D, with changes every 2 days

On day 27, media is changed to HM-E, with changes every 3 days until day 39

Media formulations:

DM: DMEM/F12, 2% Probumin, 1x NEAA, 2mM L-Alanyl-L-Glutamine, 1x Trace elements A, B, & C, 10ug/ml Human Transferrin, 50ug/ml Ascorbic acid

CDM: DM with the following added fresh daily:

8ng/ml bFGF, 200ng/ml Long R3-IGF1, 10ng/ml Activin A, 10ng/ml Heregulin β1

HM-A: Low Glucose DMEM, 2mM L-Alanyl-L-Glutamine, 50ug/ml Ascorbic acid, 0.45mM

1-Thioglycerol, 1% B27, 50ng/ml BMP4, 50ng/ml Fgf10

HM-B: HM-A with 40ng/ml bFgf replacing Fgf10

HM-C: Low Glucose DMEM:F12 (75:25), 2mM L-Alanyl-L-Glutamine, 50ug/ml Ascorbic acid, 0.45mM 1-Thioglycerol, 1% B27, 0.1% Probumin, 20ng/ml HGF, 40ng/ml Dexamethasone, 20ng/ml Oncostatin M.

HM-D: HM-C with High Glucose DMEM replacing Low Glucose DMEM

HM-E: Lonza HCM (Hepatocyte Culture Media) without EGF, 1mM cAMP