

# Differentiation of Human Pluripotent Stem Cells into Pancreatic Beta-cell Precursors in a 2D Culture System

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## Materials

Name	Company	Catalog Number	Comments
15 mL, conical, centrifuge tubes	Thermo Scientific	339651	
20X TBS Tween 20	Thermo Scientific	28360	
24-well culture plates, flat bottom with lid	Costar	3524	
50 mL, conical, centrifuge tubes	Thermo Scientific	339652	
6- well culture plates, multidish	Thermo Scientific	140685	
Accutase	Stem Cell Technologies	0-7920	
Activin A	R&D	338-AC	Reconstituted in 4 mM HCl
Anti NKX6.1 antibody, mouse monoclonal	DSHB	F55A12-C	Diluted to 1:100 for flow-cytometry and 1:2000 for immunostaining
Anti-PDX1 antibody, guinea pig polyclonal	Abcam	ab47308	Diluted to 1:100 for flow-cytometry and 1:1000 for immunostaining
B27 minus Vit A	ThermoFisher	12587010	
Bovine serum albumin, heat shock fraction, fatty acid free	Sigma	A7030	
CHIR 99021	Tocris	4423	Reconstituted in DMSO
DMEM, high glucose	ThermoFisher	41965047	
Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	Invitrogen	A10037	
Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488		A-21206	
DPBS 1X	ThermoFisher	14190144	
EGF	ThermoFisher	PHG0313	Reconstituted in 0.1% BSA in PBS
FGF10	R&D	345-FG	Reconstituted in PBS
Glucose	Sigma Aldrich	G8644	
Hoechst 33258	Sigma	23491-45-4	
Inverted microscope	Olympus	IX73	
KnockOut DMEM/F-12 (1X)	Gibco	12660-012	

KnockOut SR serum replacement	Gibco	10828-028	
L-Ascorbic acid (vitamin C)	Sigma	A92902	Reconstituted in distilled water
Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix	Corning	354230	Aliquot the thawed stock and freeze at -20C.
MCDB131	ThermoFisher	10372019	
Mouse anti-SOX17	ORIGENE	CF500096	Diluted to 1:100 for flow-cytometry and 1:2000 for immunostaining
mTeSR Plus	Stem Cell Technologies	85850	Mix the basal media with supplement. Aliquot and store at -20 °C for longer time or at 4 °C for instant use
Nalgene filter units, 0.2 µm PES	ThermoFisher	566-0020	
Nicotinamide	Sigma	72340	Reconstituted in distilled water
NOGGIN	R&D	6057-NG	Reconstituted in 0.1% BSA in PBS
Paraformaldehyde solution 4% in PBS	ChemCruz	sc-281692	
Penicillin-Streptomycin (10,000 U/ mL)	ThermoFisher	15140122	
Portable vacuum aspirator			
Rabbit anti-FOXA2	Cell signaling technology	3143	Diluted to 1:100 for flow-cytometry and 1:500 for immunostaining
Retinoic Acid	Sigma Aldrich	R2625	Reconstituted in DMSO
Rock inhibitor (Y-27632)	ReproCell	04-0012-02	Reconstituted in DMSO
Round Bottom Polystyrene FACS Tubes with Caps, STERILE	Stellar Scientific	FSC-9010	
SANT-1	Sigma Aldrich	S4572	Reconstituted in DMSO
Sodium bicarbonate	Sigma	S5761-500G	
StemFlex	ThermoFisher	A3349401	Mix the basal media with supplement. Aliquot and store at -20 °C for longer time or at 4 °C for instant use
TALI Cellular Analysis Slide	Invitrogen	T10794	
Tali image-based cytometer automated cell counter	Invitrogen	T10796	
Triton X-100	Sigma	9002-93-1	
TrypLE 100 mL	ThermoFisher	12563011	
Tween 20	Sigma	P2287	
UltraPure 0.5 M EDTA, pH 8.0	Invitrogen	15575-038	