

Materials List for:

Measuring Relative Insulin Secretion using a Co-Secreted Luciferase Surrogate

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Materials

Name	Company	Catalog Number	Comments
Cell culture materials			
rIns-GLuc stable MIN6 cells			Parental MIN6 cell line stably expressing pcDNA3.1+rInsp-Ins-eGLuc and maintained in 250 ug/ml G418
DMEM	Sigma	D6429	4.5 g/L glucose media
fetal bovine serum, heat-inactivated	Sigma	F4135	
Penicillin/Streptomycin	Thermo-Fisher Scientific	SV30010	
beta-mercaptoethanol	Thermo-Fisher Scientific	BP 176-100	
glutamine	Thermo-Fisher Scientific	BP379-100	
Trypsin-EDTA	Sigma	T3924-500	
G418	Gold Biotechnology	G418-10	Stock solution 250 mg/mL in water. Freeze aliquots at -20C.
T75 tissue culture flasks	Fisher Scientific	07-202-000	
96 well tissue culture plates	Celltreat	229196	
Reagent reservoirs (50 mL)	Corning	4870	
Name	Company	Catalog Number	Comments
Secretion assay reagents			
BSA (RIA grade)	Thermo-Fisher Scientific	50-146-952	
D-(+)-Glucose	Sigma	G8270-1KG	
KCl	Thermo-Fisher Scientific	P217-500	
NaCl	Thermo-Fisher Scientific	S271-3	
Hepes, pH 7.4	Thermo-Fisher Scientific	50-213-365	
NaHCO ₃	Thermo-Fisher Scientific	15568414	
MgCl ₂	Thermo-Fisher Scientific	M9272-500G	
CaCl ₂	Sigma	C-7902	
Name	Company	Catalog Number	Comments
Optional drugs for stimulation experiments			
Diazoxide	Sigma	D9035	Stock solution: 50 mM in 0.1N NaOH. Add equal amount of 0.1N HCl to any buffer where diazoxide is added.
epinephrine (bitartrate salt)	Sigma	E4375	Stock solution: 5 mM in water
PMA (phorbol 12-myristate)	Sigma	P1585	Stock solution: 100 μM in DMSO
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Guassia assay materials			
Disodium phosphate (Na ₂ HPO ₄)	Thermo-Fisher Scientific	S374-500	
Glycerol	Thermo-Fisher Scientific	G334	
Sodium Bromide	Thermo-Fisher Scientific	AC44680-1000	
EDTA	Thermo-Fisher Scientific	AC44608-5000	Stock solution: 0.5 M pH 8
Tris base	RPI	T60040-1000.0	Stock solution: 1 M pH 8
Ascorbic Acid	Fisher Scientific	AAA1775922	US Patent US7718389 suggested ascorbate can increase coelenterazine stability.
Na ₂ SO ₃	Sigma	S0505-250G	US Patent US8367357 suggested sulfite may decrease background due to BSA
Coelenterazine (native)	Nanolight / Prolume	3035MG	Stock solution: 1 mg/ml in acidified MeOH (2.36 mM)
OptiPlate-96, White Opaque 96-well Microplate	Perkin Elmer	6005290	Any opaque white 96 well plate should be sufficient. Clear bottom plates will also work, however some signal will be lost.
Name	Company	Catalog Number	Comments
Equipment			
Synergy H1 Hybrid plate reader or equivalent	BioTek	8041000	A plate reader with luminescence detection and 96-well plate capabilities is required.
8-channel VOYAGER Pipette (50-1250 µL)	Integra	4724	An automated multichannel pipette is extremely useful for rapid addition of luciferase reagents and plating cells in 96 well format
8-channel 200 µL pipette	Transferpette S 20-200 µL	2703710	