

Materials List for:

Transient Expression in Red Beet of a Biopharmaceutical Candidate Vaccine for Type-1 Diabetes

Mattia Santoni¹, Edoardo Bertini¹, Roberta Zampieri¹, Anna Cuccurullo¹, Mauro Commisso¹, Elisa Gecchele¹, Linda Avesani¹

¹Department of Biotechnology, University of Verona

*These authors contributed equally

Correspondence to: Elisa Gecchele at elisa.gecchele@univr.it, Linda Avesani at linda.avesani@univr.it

URL: <https://www.jove.com/video/59298>

DOI: [doi:10.3791/59298](https://doi.org/10.3791/59298)

Materials

Name	Company	Catalog Number	Comments
0.2-µm Minisart RC4 membrane filters	Sartorius-Stedim	17764	
2-mercaptoethanol	Sigma	M3148	Toxic; 4 % to make loading buffer with glycerol, SDS and Tris-HCl
4-Morpholineethanesulfonic acid (MES)	Sigma	M8250	pH 5.5
96-well plate	Sarstedt	833924	
Acetic acid	Sigma	27221	Corrosive
Acetonitrile LC-MS grade	Sigma	34967	
Acetosyringone	Sigma	D134406	Toxic – 0.1 M stock in DMSO
Agar Bacteriological Grade	Applichem	A0949	15 g/L to make LB medium (pH 7.5 with NaOH) with Yeast extract, NaCl and Tryptone
Ammonium formate	Sigma	70221	
Anti-eGFP antibody	ABCam	ab290	
Anti-GAD 65/67 antibody	Sigma	G5163	
Anti-LHCB2 antibody	Agrisera	AS01 003	
Brilliant Blue R-250	Sigma	B7920	
C18 Column	Grace	-	Alltima HP C18 (150 mm x 2.1 mm; 3 µm) Column
C18 Guard Column	Grace	-	Alltima HP C18 (7.5 mm x 2.1 mm; 5 µm) Guard Column
CalMag Grower	Peter Excel	15-5-15	Fertilizer
Carbenicillin disodium	Duchefa Biochemie	C0109	Toxic
Chemiluminescence imaging system	BioRad	1708370	ChemiDoc Touch Imaging System
Chloroform	Sigma	C2432	
Detergent	Sigma	P5927	Polysorbate 20
Fluorescence reader	Perkin-Elmer	1420-011	VICTOR Multilabel Counter
Formic acid LC-MS grade	Sigma	94318	
Glycerol	Sigma	G5516	15 % to make loading buffer with Tris-HCl, SDS and 2-mercaptoethanol
GoTaq G2 polymerase	Promega	M7841	
HCl	Sigma	H1758	Corrosive

HILIC Column	Grace	-	Ascentis Express HILIC (150 mm x 2.1 mm; particles size 2.7 µm) Column
HILIC Guard Column	Grace	-	Vision HT HILIC (7.5 mm x 2.1 mm; 3 µm) Guard Column
Horseradish peroxidase (HRP)-conjugate anti-rabbit antibody	Sigma	A6154	Do not freeze/thaw too many times
HPLC Autosampler	Beckman Coulter	-	System Gold 508 Autosampler
HPLC System	Beckman Coulter	-	System Gold 128 Solvent Module HPLC
Isopropanol	Sigma	24137	Flammable
Kanamycin sulfate	Sigma	K4000	Toxic
KCl	Sigma	P9541	2 g/L with NaCl , Na ₂ HPO ₄ and KH ₂ PO ₄ to make PBS
KH ₂ PO ₄	Sigma	P9791	2.4 g/L with NaCl , Na ₂ HPO ₄ and KCl to make PBS
Loading Buffer			
Luminol solution	Ge Healthcare	RPN2232	Prepare the solution using the ECL Prime Western Blotting System commercial kit
Lyophilizator	5Pascal	LIO5P0000DGT	
Mass Spectrometer	Bruker Daltonics	-	Bruker Esquire 6000; the mass spectrometer was equipped with an ESI source and the analyzer was an ion trap
Methanol	Sigma	32213	
MgSO ₄	Sigma	M7506	
Milk-blocking solution	Ristora	-	3 % in PBS
Na ₂ HPO ₄	Sigma	S7907	Use with NaH ₂ PO ₄ to make Sodium Phosphate buffer
NaCl	Sigma	S3014	80 g/L with KCl, Na ₂ HPO ₄ and KH ₂ PO ₄ to make PBS; 10 g/L to make LB medium (pH 7.5 with NaOH) with Yeast extract, Tryptone and Agar Bacteriological Grade
NaH ₂ PO ₄	Sigma	S8282	Use with Na ₂ HPO ₄ to make Sodium Phosphate buffer; 14.4 g/L to make PBS
NaOH	Sigma	S8045	
Nitrocellulase membrane	Ge Healthcare	10600002	
Pepsin from porcine gastric mucosa	Sigma	P7000	
Peroxidase substrate ECL	GE Healthcare	RPN2235	Light sensitive material
Pump Vacuum Press	VWR	111400000098	
Reagent A	Sigma	B9643	Use 50 parts of this reagent with 1 part of reagent B to prepare BCA working solution
Reagent B	Sigma	B9643	Use 1 part of this reagent with 50 parts of reagent A to prepare BCA working solution
Rifampicin	Duchefa Biochemie	R0146	Toxic – 25 mg/mL stock in DMSO
SDS (Sodium dodecyl sulphate)	Sigma	L3771	Flammable, toxic, corrosive-10 % stock; 3 % to make loading buffer

			with Tris-HCl, Glycerol and 2-mercaptoethanol
Sodium metabisulphite	Sigma	7681-57-4	
Sonicator system	Soltec	090.003.0003	Sonica® 2200 MH; frequency 40 khz
Syringe	Terumo	-	
Transparent fixed 300-µL insert glass tubes	Thermo Scientific	11573680	
Trizma Base	Sigma	T1503	Adjust pH with 1N HCl to make Tris-HCl buffer, use 1,5M Tris-HCl (pH 6.8) to make loading buffer with SDS, Glycerol and 2-mercaptoethanol
Tryptone	Formedium	TRP03	10 g/L to make LB medium (pH 7.5 with NaOH) with Yeast extract, NaCl and Agar Bacteriological Grade
Vacuum concentrator	Heto	3878 F1-3	Speed-vac System
Water LC-MS grade	Sigma	39253	
Yeast extract	Sigma	Y1333	5 g/L to make LB medium (pH 7.5 with NaOH) with Tryptone, NaCl and Agar Bacteriological Grade