

Detecting Virus and Salivary Proteins of a Leafhopper Vector in the Plant Host

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Citation

 Wang, Y., Wang, X., Li, Z., Chen, Q. Detecting Virus and Salivary Proteins of a Leafhopper Vector in the Plant Host. *J. Vis. Exp.* (175), e63020, doi:10.3791/63020 (2021).

Date Published

September 14, 2021

DOI

10.3791/63020

URL

jove.com/video/63020

Materials

Name	Company	Catalog Number	Comments
Reagents			
Tris base	Roche	D609K69032	For 5×Tris-glycine buffer and 10×TBS buffer preparation
glycine	Sigma-Aldrich	WXBD0677V	For 5×Tris-glycine buffer preparation
SDS	Sigma-Aldrich	SLCB4394	For 5×Tris-glycine buffer preparation
NaCl	Sinopharm Chemical Reagent Co., Ltd	10019318	For 10×TBS buffer preparation
KCl	Sinopharm Chemical Reagent Co., Ltd	10016318	For 10×TBS buffer preparation
β-mercaptoethanol	Xiya Reagent	B14492	For 4× protein sample buffer preparation
bromophenol blue	Sigma-Aldrich	SHBL3668	For 4× protein sample buffer preparation
glycerol	Sinopharm Chemical Reagent Co., Ltd	10010618	For 4× protein sample buffer preparation
methanol	Sinopharm Chemical Reagent Co., Ltd	10014118	For transfer buffer preparation
Tween 20	Coolaber SCIENCE&TeCHNoLoGY	CT30111220	For TBST preparation
non-fat dry milk	Becton.Dickinso and company	252038	For membrane blocking, antibodies dilution
goat anti-rabbit IgG	Sangon Biotech	D110058-0001	Recognition of the primary antibody
ECL Western kit	ThermoFisher Scientific	32209	Chemiluminescent substrate
nitrocellulose membrane	Pall Corporation	25312915	For proteins transfer
Buffers and Solutions			
Buffer	Composition		Comments/Description
5×Tris-glycine buffer	15.1 g Tris base 94 g glycine 5 g SDS in 1 L sterile water		Stock solution
1×Tris-glycine buffer	200 mL of 5×Tris-glycine buffer 800 mL sterile water		Work solution, for SDS-PAGE
10×Tris-buffered saline (TBS) buffer	80 g NaCl		Stock solution

	30 g Tris base 2 g KCl in 1 L sterile water		
TBS with Tween 20 (TBST) solution	100 mL 10×TBS solution 3 mL Tween 20 900 mL sterile water		Work solution
4× protein sample buffer	8 g SDS 4 mL β-mercaptoethanol 0.02 g bromophenol blue 40 mL glycerol in 40 mL 0.1 M Tris-HCl (pH 6.8)		For protein extraction
Transfer buffer	800 mL Tris-glycine buffer 200 mL methanol		For protein transfer
Instruments			
Bromophenol blue	Sigma-Aldrich	SHBL3668	For 4x protein sample buffer preparation
Constant temperature incubator	Ningbo Saifu Experimental Instrument Co., Ltd.	PRX-1200B	For rearing leafhoppers
Electrophoresis	Tanon Science & Technology Co., Ltd.	Tanon EP300	For SDS-PAGE
Electrophoretic transfer core module	BIO-RAD	1703935	For SDS-PAGE
glycerol	Sinopharm Chemical Reagent Co., Ltd	10010618	For 4x protein sample buffer preparation
glycine	Sigma-Aldrich	WXBD0677V	For 5x Tris-glycine buffer preparation
goat anti-rabbit IgG	Sangon Biotech	D110058-0001	Recognition of the primary antibody
High-pass tissue grinding instrument	Shanghai Jingxin Industrial Development Co., Ltd.	JXFSIPRP-24	For grinding plant tissues
KCl	Sinopharm Chemical Reagent Co., Ltd	10016318	For 10x TBS buffer preparation
methanol	Sinopharm Chemical Reagent Co., Ltd	10014118	For transfer buffer preparation
Mini wet heat transfer trough	BIO-RAD	1703930	For SDS-PAGE
NaCl	Sinopharm Chemical Reagent Co., Ltd	10019318	For 10x TBS buffer preparation
nitrocellulose membrane	Pall Corporation	25312915	For proteins transfer
non-fat dry milk	Becton.Dickinso and company	252038	For membrane blocking, antibodies dilution
Pierce ECL Western kit	ThermoFisher Scientific	32209	Chemiluminescent substrate
Protein color instrument	GE Healthcare bio-sciences AB	Amersham Imager 600	For detecting proteins
SDS	Sigma-Aldrich	SLCB4394	For 5x Tris-glycine buffer preparation
Tris base	Roche	D609K69032	For 5x Tris-glycine buffer and 10×TBS buffer preparation
Tween 20	Coolaber SCIENCE&TeCHNoLoGY	CT30111220	For TBST preparation
Vertical plate electrophoresis tank	BIO-RAD	1658001	For SDS-PAGE
Water bath	Shanghai Jinghong Experimental equipment Co., Ltd.	XMTD-8222	For boil the protein samples
β-mercaptoethanol	Xiya Reagent	B14492	For 4x protein sample buffer preparation