

Materials List for:

# Thin-layer Chromatographic (TLC) Separations and Bioassays of Plant Extracts to Identify Antimicrobial Compounds

Isabelle A. Kagan<sup>1</sup>, Michael D. Flythe<sup>1</sup>

<sup>1</sup>Forage-Animal Production Research Unit, Agricultural Research Service, United States Department of Agriculture

Correspondence to: Isabelle A. Kagan at [isabelle.kagan@ars.usda.gov](mailto:isabelle.kagan@ars.usda.gov)

URL: <https://www.jove.com/video/51411>

DOI: [doi:10.3791/51411](https://doi.org/10.3791/51411)

## Materials

Name	Company	Catalog Number	Comments
Silica F <sub>254</sub> TLC plates, aluminum-backed, 0.2 mm thickness, 20 cm x 20 cm	EMD Chemicals	5554/7	These plates are coated with silica that contains an indicator fluorescing at 254 nm. Compounds absorbing at that wavelength appear dark on a fluorescent green background. Alternative sources include Analtech, Selecto Scientific, Fluka. Adsorbents other than silica may be needed. Plastic-backed plates may be suitable, depending on the solvents to be used.
Sharp, heavy-duty scissors	any sewing supply company	similar to Fiskars 175800-1002	For cutting TLC plates. A paper cutter with a sharp blade can be used as well. Do not inhale silica dust.
Drying oven at 100 °C (mechanical convection)	Thermo Scientific	PR305225M	Quincy Lab, Inc, Chicago, IL ( <a href="http://www.quincylab.com">www.quincylab.com</a> ); Cascade Technical Sciences, Hillsboro, OR ( <a href="http://www.cascadetek.com">www.cascadetek.com</a> )
TLC chamber	Kimble Chase	416180-0000	Alternative sources: Aldrich. Pyrex beakers or preserving jars can be used for small plates (i.e. 5 cm x 10 cm). Cover with aluminum foil (jar lids may contain material extractable by solvent vapors).
50 µl Syringe with flat needle tip	Hamilton	80965	For loading amounts of standard or sample exceeding 5-10 µl. Alternative sources are equivalent.
Micropipettes	Drummond	2-000-001	For loading small amounts of standards or samples. Alternative sources: VWR. Also, Pasteur pipets can be stretched to a thinner diameter with a butane torch.
Filter paper (#1 grade)	Whatman	1001 917	Serves as a chamber wick. Other grades of filter paper are OK. This size can be trimmed for the chambers holding 20 cm x 20 cm plates.
Beaker tongs	Fisher Scientific	15-186	For putting plates in and out of a large TLC chamber. Alternate sources: VWR
Flat-edge forceps	Fisher Scientific	10-275	For putting plates in and out of a small chamber. Alternate sources: VWR
Small portable UV lamp with 4 W or 6 W bulbs for short- and long-	Ultraviolet Products	95-0271-01	Alternate sources: Spectronics Corporation ( <a href="http://www.spectroline.net">www.spectroline.net</a> )

wave UV light illumination (254 and 365 nm, respectively)			
Viewing cabinet for use with hand-held UV lamp	Ultraviolet Products	Chromato-Vue C-10E	UV-active bands are more easily circled if plates can be set in here. Alternate sources: Spectronics Corporation.
Photodocumentation system with overhead UV lamp and visible lamp	Kodak	Gel Logic 200	Alternate sources: Ultraviolet Products (www.uvp.com). See protocol for homemade alternative.
Anaerobic Chamber, Type A, Vinyl	Coy	7150000	This chamber is appropriate for anaerobic bacteria, like <i>Clostridium sticklandii</i> , as described. However, growth conditions must be tailored to organism used in the assay. A biosafety cabinet and other precautions should be taken if pathogenic organisms are used. Alternate sources: Anaerobe Systems, BioRad, Plas Labs, others
Tetrazolium red	Sigma-Aldrich	T8877	Alternate sources: MP Biomedicals, Santa Cruz Biotechnology, Alfa Aesar
<b>Ingredients for HAB media</b>			
Pyridoxamine · 2HCl	Sigma-Aldrich	P9380	For this and for all the other reagents in this table, alternative sources are equivalent.
Riboflavin	Sigma-Aldrich	R4500	
Thiamine HCl	Sigma-Aldrich	T3902	
Nicotinamide	Sigma-Aldrich	N3376	
Calcium D-Pantothenate	Sigma-Aldrich	C8731	
Lipoic Acid	Sigma-Aldrich	T5625	
p-Aminobenzoic acid	Sigma-Aldrich	A9878	
Folic acid	Sigma-Aldrich	F8798	
Biotin	Sigma-Aldrich	B4639	
Cobalamine	Sigma-Aldrich	C3607	
Pyridoxal HCl	Sigma-Aldrich	P9130	
Pyridoxine	Sigma-Aldrich	P5669	
EDTA	Sigma-Aldrich	E6758	
Iron sulfate · 7H <sub>2</sub> O	Sigma-Aldrich	F8263	
Zinc sulfate · 7H <sub>2</sub> O	Sigma-Aldrich	Z0251	
Manganese chloride · 4H <sub>2</sub> O	Sigma-Aldrich	M8054	
Boric acid	Sigma-Aldrich	B6768	
Cobalt chloride · 6H <sub>2</sub> O	Sigma-Aldrich	C8661	
Copper chloride · 2H <sub>2</sub> O	Sigma-Aldrich	459097	
Nickel chloride · 6H <sub>2</sub> O	Sigma-Aldrich	203866	
Sodium molybdate · 2H <sub>2</sub> O	Sigma-Aldrich	331058	
Trypticase (Pancreatic digest of casein)	Thermo Fisher	B11921	
Potassium phosphate monobasic anhydrous	Thermo Fisher	P284	
Sodium carbonate · H <sub>2</sub> O	Thermo Fisher	S636	
Agar	Thermo Fisher	50841063	

Magnesium sulfate · 6H <sub>2</sub> O	Thermo Fisher	7791-18-6	
Calcium chloride · 2H <sub>2</sub> O	Thermo Fisher	BP510	
Cysteine HCl	Thermo Fisher	19464780	
Potassium phosphate dibasic anhydrous	Thermo Fisher	P290	
Sodium chloride	Thermo Fisher	BP358	