

FN3PT. National Federation of Seed Potato Growers. 43-45 rue de Naples, 75008 Paris, France



INRA, National Institute for Agronomic Research UMR BiO3P1099, 35653 Le Rheu Cedex

Crystal violet pectate single layer medium preparation **SL-CVP**_{AG366}

(From V. Hélias, P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon, 2011. Two new effective semi-selective crystal violet pectate (CVP) media for isolation of Pectobacterium and Dickeya. Plant Pathology, 61, 339-345; valerie.helias@rennes.inra.fr)

Crystal	violet	mix	
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CaCl ₂ .2H ₂ O	1.02 g

•	Tryptone	1.0 g
•	Tri-sodium citrate	5.0 g
•	Na NO3	2.0 g
•	Crystal violet (0.1%) *	1.5 ml
•	Agar	4.0 g

Pectin mix

In 500 ml distilled water:

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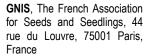
•	NaOH (5M)	2.8 ml
•	Pectin Dipecta (ref AG366)	18.0 g

- Introduce ingredients of both mixes in the order of the component list.
- Dissolve each ingredient of the crystal violet mix by stirring the medium before adding the following one.
- Stir the pectin mix and heat it up (80-100°C) to allow the pectin to be dissolved avoiding formation of lumps.
- Sterilise both mixes at 120°C for 15 min
- Pour slowly the crystal violet mix into the pectin mix while still hot, by gently (to avoid bubble formation) stirring the medium (using a magnetic stirrer).
- Verify the pH being between 6.8-7.4
- Distribute in Petri dishes the resulting medium immediately after autoclaving.
- Allow them drying to eliminate condensation in a laminar flow cabinet
- Store the CVP₂₀₀₅ medium at 4°C.
- Petri dishes are dried with the lids ajar in the laminar flow cabinet to eliminate the condensation before use.

A Research Program funded by French Potato Professionals:

Pectin source distributed by:







CNIPT, The ware potato professionals (growers, cooperatives, retailers...), 43-45 rue de Naples, 75008 Paris, France



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^{*} Please note that this amount is a correction to the original publication, which incorrectly described the use of 1.0% crystal violet for the formulation of SL-CVP.



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Crystal violet pectate double layer medium preparation **DL-CVP**_{AG366}

(From **V. Hélias**, P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon, 2011. Two new effective semi-selective crystal violet pectate (CVP) media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61, 339-345; valerie.helias@rennes.inra.fr)

Basal layer medium

•	CaCl ₂ , 2H ₂ O	5.5 g
•	Tryptone	1 g
•	Crystal violet 0,1%	1.5 ml
•	NaNO3	1.6 g
•	Agar	15 g
•	Distilled water	1000 ml

- Introduce ingredients in the order of the component list.
- Dissolve each component by stirring the medium before adding the following one.
- Sterilise the basal layer medium at 120°C for 15 min
- Pour the medium in Petri dishes (15ml / dishes) in a laminar flow cabinet
- Allow them to set before pouring the overlayer medium

Overlayer medium

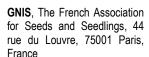
•	EDTA 5,5%,(pH 8.0)	2 ml
•	NaOH 5M	4.8 ml
•	Distilled water	800 ml
•	Pectin Dipecta (ref AG366)	20 g

- Heat up (80-100°C) and stir the overlayer mix to allow the pectin to be dissolved
- Distribute the medium in 2 X 400 ml bottles before autoclaving (120°C for 15 min).
- Verify the pH (6.8-7.4) before pouring
- Pour the medium (7 ml) when the basal layer is dry
- Petri dishes are dried in a laminar flow cabinet for 24 to 48 hours
- The DL-CVP_{AG366} medium is stored at 4°C until used
- Petri dishes are dried with the lids ajar in the laminar flow cabinet to eliminate the condensation before use.

A Research Program funded by French Potato Professionals:

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Liquid Enrichment Medium **LEM**_{AG366} preparation

(From **V. Hélias**, P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon, 2011. Two new effective semi-selective crystal violet pectate (CVP) media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61, 339-345; <u>valerie.helias@rennes.inra.fr</u>)

Liquid Medium composition

•	NaOH 5N	20 µl
•	MgSO₄, 7H₂O	0.0375 g
•	$(NH)_4SO_4$	0.1 g
•	K ₂ HPO4	0.1 g
•	Pectin Dipecta (ref AG366)	0.17 g
•	H2O	gsp 100 ml

- Mix the NaOH solution in water. Add the component in the following order: MgSO₄, (NH)₂SO₄ and K₂HPO₄ and finally the pectin
- Sterilise both mixes at 120°C for 15 min
- Verify that the pH is comprised between 6.8 et 7.4
- Store the LEM_{AG366} at 4°C.
- Enrichment procedure is applied by adding 10 volumes of LEM_{AG366} to 1 volume of sample to be tested

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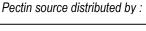
GNIS, The French Association for Seeds and Seedlings, 44 rue du Louvre, 75001 Paris, France



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