

VERSION 2

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Protocol status: In development
We are still developing and optimizing this protocol

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ABSTRACT

For the growth and maintenance of Cochliobolus carbonum and Cochliobolus victoriae

PROTOCOL integer ID: 81948

MATERIALS

Trace Minerals

Boric Acid (H3BO3) CAS:<u>10043-35-3</u> Cupric Sulfate (CuSO4) CAS: <u>7758-98-7</u> Potassium Iodide (KI) CAS:<u>7681-11-0</u>

Manganese (II) sulfate monohydrate MnSO₄ * H2O CAS: <u>10034-96-5</u> Sodum permanganate monohydrate NaMoO₄ *H2O CAS: <u>79048-36-5</u>

Zinc sulfate heptahydrate $ZnSO_4*7H_2O$ CAS: $\underline{7446-20-0}$ Iron (III) chloride hexahydrate $FeCl_3*6H_2O$ CAS: $\underline{10025-77-1}$

Salts

Calcium nitrate tetrahydrate $Ca(NO_3)_2 * 4 H_2O CAS: 13477-34-4$

Potassium Phosphate monobasic KH2PO4 CAS: 7778-77-0

Magnesium Sulfate heptahydrate MgSO4 * 7H2O CAS: 10034-99-8

Sodium Chloride NaCl CAS: 7647-14-5

Media

Yeast Extract CAS: 8013-01-2 (some people report differences between difference sources)

Casein Digests: Still a bit of work to do here to determine, which of these is best (including new plant-based sources). Big differences in prices between different digest types.

Acid hydrolysed Casein CAS: 65072-00-6

Peptone from casein, tryptic digest OR pancreatic digest (tryptone) CAS: 91079-40-2

Make Micronutrients Solution

1 9 mg H₃BO₃

58.5 mg CuSO₄ *5H₂O

1.95 mg KI (Potassium Iodine)

9 mg MnSO₄

7.6 mg NaMoO₄

822 mg ZnSO₄ * 6 H₂O

139.8 mg FeCl₃ * 6H₂O

in 300 mL ddH20 and filter sterilise

Citation:

Heterokaryosis and Parasexuality in the Fungus Ascochyta Imperfecta Author(s): K. E. Sanderson and A. M. Srb Source: American Journal of Botany, Jan., 1965, Vol. 52, No. 1 (Jan., 1965), pp. 72-81 Published by: Wiley Stable URL: https://www.jstor.org/stable/2439977

Make 100x Salt Solutions A and B

2 100X Salt Solution A

10g Ca(NaO₃)₂ * 4 H₂O 100 mL ddH₂O

Autoclave

Citation:

Leach, J., Lang, B. R. & Yoder, O. C. *Microbiology***128**, 1719-1729, doi:https://doi.org/10.1099/00221287-128-8-1719 (1982).

3 100X Salt Solution B

 $2 \text{ g } \text{KH}_2\text{PO}_4*7 \text{ H}_2\text{O}$ 1.5g NaCl

100 mL H20 pH 5.3

Autoclave

Citation:

Leach, J., Lang, B. R. & Yoder, O. C. *Microbiology***128**, 1719-1729, doi:https://doi.org/10.1099/00221287-128-8-1719 (1982).

Make Complete Medium (CM) or Complete Medium Xylose (...

4 Complete Medium Base

10 g glucose OR xylose (substitute glucose for xylose for CMX medium)

1 g Yeast Extract

0.5 g Acid-hydrolysed Casein

0.5 g Enzyme-hydrolysed casein

20g Agar

10 mL Salt A

10 mL Salt B

Make up to 1000 mL with ddH20 and Autoclave

After autoclaving add:

1 mL sterilised micronutrient solution

Citation:

Leach, J., Lang, B. R. & Yoder, O. C. *Microbiology***128**, 1719-1729, doi:https://doi.org/10.1099/00221287-128-8-1719 (1982).

Complete Medium for Sporulation

5 Complete Medium Base

0.5 g glucose OR xylose (substitute glucose for xylose for CMX medium)

20 g Sorbose

1 g Yeast Extract

0.5 g acid hydrolysed casein

0.5 g enzyme hydrolysed casein

20g Agar

10 mL Salt A

10 mL Salt B

Make up to 1000 mL with ddH20 and Autoclave

After autoclaving add:

1 mL sterilised micronutrient solution