

Rye agar (for Mycelium growth)

Preparing rye concentrate

- 600 g rye grain (biological).
- Disinfect for 10 minutes in 1 litre 2% Sodium-hypochloride.
- Wash 4 to 5 times with distilled water using a sieve.
- Bring the rye over to a dry clean tub and add 500 ml distilled water.
- Cover the tub with Saran wrap and a dark cloth.
- Let the rye germinate at room temperature for 36-48 hours.
- Pour off the liquid and wash the grain twice with distilled water.
- Add 1 litre tap water and blend for 20 seconds.
- Pour the suspension in two 1 litre flasks
- Incubate in waterbath (or incubator) for 3 hours at 50°C.
- Filter through sieve and discard grains (save suspension, about 1200 ml).
- Bring end volume up to 2 litre with distilled water.
- Store Rye concentrate at -20°C

20 % Rye Agar (RA)

- Take 200 ml rye concentrate.
- Add 20 g saccharose (table sugar) and 15 g microagar.
- Bring end volume with distilled water to 1 litre.
- Autoclave 20 minutes 120°C.

20 % Rye Agar for oospore production

- Take 200 ml rye concentrate.
- Add 20 g saccharose (table sugar), 0,1 g β -sitosterol and 15 g microagar.
- Bring end volume with tap water to 1 litre.
- Autoclave 20 minutes 120°C.

20 % Rye Agar Selective Media (RASM)

- Take 200 ml rye suspension.
- Add 20 g saccharose (table sugar) and 15 g microagar.
- Bring end volume with distilled water to 1 litre.
- Autoclave 20 minutes 120°C.
- Let the medium cool down to luke-warm (ca. 40°C).
- Add 10 ml antibiotic mix, and mix thoroughly.
- Poor the plates immediately.

Antibiotic mix:

Material:

- Ampicillin 250 mg/ml H₂O, filter sterilised or dissolved in ethanol 70 – 96%
- Rifampicin 12 mg/ml DMSO
- Oxytetracycline 8 mg/ml ethanol
- Pimaricin 2 mg/ml methanol

For the antibiotic mix add together: 4 ml Ampicillin, 12,5 ml Rifampicin, 6,25 ml Oxytetracyclin, 25 ml Pimaricin and 2,25 ml H₂O. (Store at –20°C)

20% Rye Agar Selective Medium for fungal infections

- Take 200 ml rye suspension.
- Add 20 g saccharose (table sugar) and 15 g microagar.
- Bring end volume with distilled water to 1 litre.
- Autoclave 20 minutes 120°C
- Let the medium cool down to luke-warm (ca. 40°C).
- Add the antibiotic mix (Hohl, 1991 #3726).

Antibiotics	
Vancomycin	100 mg/l
Polymixin B	500 mg/l

Ampicillin	200 mg/l
Rifampicin	20 mg/l
PCNB (75% WP)	67 mg/l
Benlate (50% WP)	100 mg/l

- mix thoroughly.
- Poor the plates immediately.

Pea broth/ Pea agar (for standard mycelium growth)

- Autoclave (15 minutes 120°C) 120 g frozen peas (supermarket) in 1 litre distilled water.
- Strain peas from pea broth using 4 layers of cheese clothe squeezing gently to remove all excess liquid from peas.
- Bring volume of broth to 1 litre.
- For pea agar add 15 g agar to 1 litre broth.
- Autoclave for 20 minutes 120°C.

V8-medium (for mycelium growth)

- Combine 200 ml V8 juice and 800 ml tapwater.
- Add 2 g CaCO₃ and 0.05 g β-sitosterol and mix well.
- Add 15 g agar.
- Autoclave for 20 minutes at 120 °C.
- Stir medium well before poring the plates in order to resuspent the CaCO₃.

Modified Plitch medium (minimal medium for mycelium growth)

- Prepare for 1 litre medium:
 - 0.5 g KH_2PO_4
 - 0.25 g $\text{Mg}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$
 - 1.0 g L- aspergine
 - 0.5 g Yeast extract
 - 0.01 g β -sitosterol
 - 25.0 g Glucose
 - 0.001 g Thiamine
 - 15 g microagar
- Bring volume to 1 litre with Milli Q water
- Autoclave for 20 minutes at 120 °C.