Technical Data Sheet

Wallerstein Differential Broth (WLD) – 2mL Liquid Media Ampoules
Cat. No. MHA000P2D

This medium is recommended for the growth of bacteria in low pH environments such as in the brewing industry.

Mode of Action
Wallerstein Differential Broth is used in the brewing industry and industrial fermentations to detect and enumerate bacteria present in small numbers in a mixed flora sample. Cycloheximide (acidione) inhibits most yeasts and molds allowing bacteria to grow.

Typical Composition (per liter of purified water)

Pancreatic Digest of Casein	5.0 g	Ferric Chloride	2.5 mg
Yeast Extract	4.0 g	Manganese Sulfate	2.5 mg
Dextrose	50.0 g	Bromocresol Green	22 mg
Monopotassium Phosphate	0.55 g	Acidione	4 mg
Potassium Chloride	425 mg		
Calcium Chloride	125 mg		
Magnesium Sulfate	125 mg		

Application
1. Collect the liquid sample in a sterile container. The sample should be a 100 ml minimum.
2. Invert one Wallerstein Differential Broth ampoule 2 to 3 times. Open the ampoule. Remove the lid of a petri dish and carefully pour the contents equally onto the absorbent pad.
3. Set up the membrane filtration apparatus. Use sterile forceps to put the membrane filter in the assembly. The grid side is up.
4. Invert the sample / diluted sample for approximately 30 seconds to thoroughly mix the sample.
5. Pour the sample / diluted sample into the funnel. If the volume is less than 20ml, add 10 ml of sterile buffered dilution water to the funnel.
6. Apply the vacuum until the funnel is empty. Then stop the vacuum.
7. Rinse the funnel with 20ml to 30ml of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.
8. Stop the vacuum when the funnel is empty. Remove the funnel from the assembly. Use sterile forceps to lift the membrane filter.
9. Put the membrane filter on the absorbent pad. Let the membrane filter bend and fall equally across the absorbent pad to make sure that the air bubbles are not trapped below the filter.
10. Secure the lid on the petri dish and invert the dish.
11. Incubate the inverted petri dish for 48-72 hours at 30-35° C.
12. Remove the petri dish from the incubator. Use a microscope to count the number of bacteria colonies on the membrane filter.
13. Interpret and report the results.

Results Reporting
Report the colony density as the number of colonies in 100ml of sample. If there's more than 200 colonies, dilute the sample and use the diluted sample in the test procedure.

Colonies in 100ml = Colonies counted / ml of sample x 100**.**

Storage and Shelf Life
The product can be used until the expiry date if the unopened ampoules are stored sealed in the aluminum foil bag at 2 – 10°C.

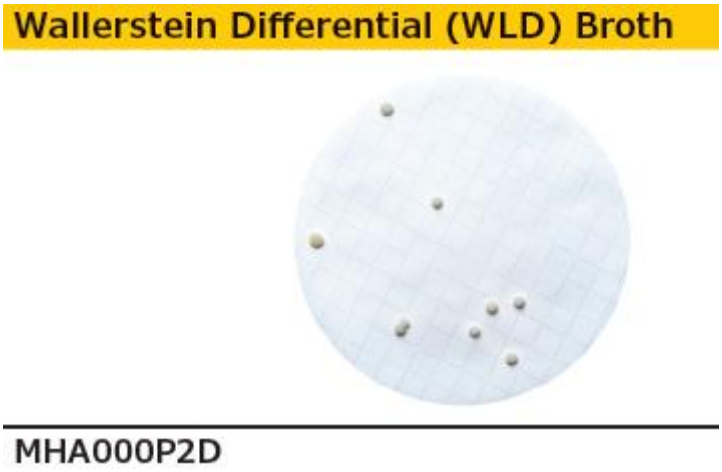
Disposal
Please dispose of used culture medium in accordance with local regulations (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Function	Control Strains	Incubation	Reference Medium	Method of Control	Expected Results
Productivity	Acetobacter aceti ATCC® 15973	48-72 hours at 30-35° C	Previously validated batch of Wallerstein Differential Broth	Quantitative	Recovery 85-115% Characteristic colonies
	Lactobacillus fermentum				
Selectivity	E. Coli			Qualitative	Growth of Characteristic Colonies
	Saccharomyces Cerevisiae				Growth inhibited

Please refer to the actual batch specific certificate of analysis.

A. aceti colonies small green-blue colonies.
Escherichia coli colonies are small green-blue colonies.
L. fermentum colonies are small green-blue colonies.
Cycloheximide resistant yeast are creamy, green white.



Ordering Information

Product	Cat. No.	Pack size
Wallerstein Differential Broth (WLD)	MHA000P2D	50 x 2 mL plastic ampoules

Literature

Compendium of methods for the microbiological examination of foods. American Public Health Association. Washington DC. 2001

Green SR and Gray PP (1950): Paper read at the American Society of Brewing Chemists Meeting. Wallerstein Laboratories Communications. Vol 12, Page 43.

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