Technical Data Sheet

Wallerstein Nutrient Broth (WLN) – 2mL Liquid Media Ampoules
Cat. No. MHA000P2N

This medium is recommended for the isolation and cultivation of yeasts, molds and bacteria that grow in a low pH environment.

Mode of Action
Wallerstein Nutrient Broth is used by the beverage industry to detect bacteria, yeast and mold in wines, beer and other fermentation products. Yeast extract is a source of trace elements, vitamins and amino acids. Peptone provides nitrogen, amino acids and carbon. Dextrose is the source of carbohydrate. Monopotassium phosphate buffers the media. Potassium chloride, calcium chloride, and ferric chloride are essential ions to help to maintain osmotic balance. Magnesium sulfate and manganese sulfate are sources of divalent cations. Bromocresol green is the pH indicator. The WL Nutrient medium plate incubated under aerobic conditions enables the growth of mainly yeast colonies, acetic acid bacteria, flavobacterium, proteus and thermophilic bacteria and under anaerobic condition enables the growth of lactic acid bacteria and Pediococcus. Bacterial colonies can appear as non-pigmented to white, with a smooth or rough texture. Yeast appear as creamy, white larger colonies.

Typical Composition (per liter of purified water)

Pancreatic Digest of Casein	5.0 g	Ferric Chloride	2.5mg
Yeast Extract	4.0 g	Manganese Sulfate	2.5mg
Dextrose	50.0 g	Bromocresol Green	22.0mg
Monopotassium Phosphate	0.55 g		
Potassium Chloride	425.0mg		
Calcium Chloride	125.0mg		
Magnesium Sulfate	125.0mg		

Application
1. Collect the liquid sample in a sterile container. The sample should be a 100 ml minimum.
2. Invert one Wallerstein Nutrient Broth (WLN) 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 (35°C for bacteria, 30°C for yeast and molds).
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.

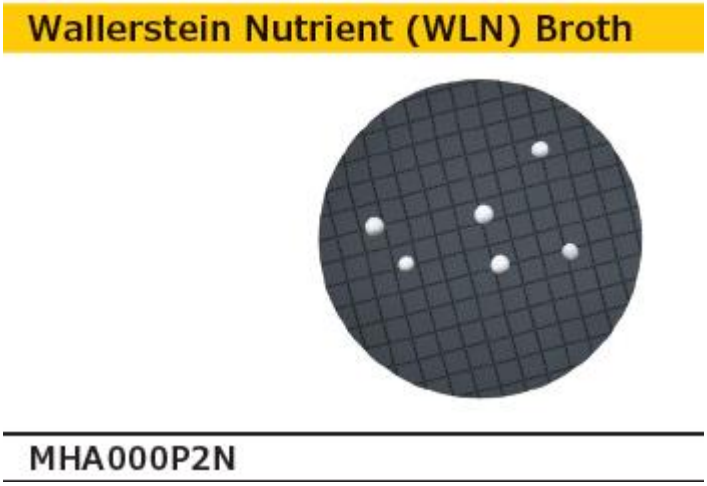
Disposal
Please dispose of used culture medium in accordance with local regulations (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Function	Control Strains	Incubation	Reference Medium	Method of Control	Expected Results
Productivity	Lactobacillus fermentum ATCC® 9338	48-72 hours at 30-35° C	Previously validated batch of WLN Broth	Quantitative	Recovery 85-115% Characteristic colonies
	Saccharomyces cerevisiae ATCC® 9763 WDCM 00058				

Please refer to the actual batch specific certificate of analysis.

Yeast appear as creamy, white larger colonies.
Bacteria can appear non-pigmented to white with smooth or rough textures.



Ordering Information

Product	Cat. No.	Pack size
Wallerstein Nutrient (WLN) Broth	MHA000P2N	50 x 2 mL plastic ampoules

Literature
MacFaddin JF, Media for Isolation Cultivation Identification Maintenance of Medical Bacteria, Volume 1, Williams and Wilkins, Baltimore, 1985.

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