User manual

NBB®-B (Product No. 2.04710.782)

1. Information

Nutrient broth for the detection of
Beer-spoiling micro organisms (esp. Lactobacilli, Pediococci, Pectinatus and Megasphaera)
in yeast samples, yeast turbid beer samples, as well as for membrane and swab samples.

NBB®-Broth (NBB®-B) (pH 5.8 ± 0.1) is a ready-to-use broth for a fast and safe detection of beer spoiling microorganisms in the brewing industry. Due to the selectivity of the NBB®-media the harmless accompanying flora as the contained yeast will be inhibited while beer spoilers are supported by a special growth component. The yeast inhibitor was selected to operate after a short initial period, time enough for the yeast to start fermentation. This provides a favourable CO_2-enviroment in the sample for an anaerobic incubation. Therefore an anaerobic incubation while working with yeast samples is not necessary.
The NBB®-B is designed for the microbiological analysis of yeast samples but can also be used for sediments of unfiltered beer, as a culture media for membrane filters, as an enrichment media for trace contaminations for further analyses.

2. Handling

Required Material
Microbiological workbench
Bunsen burner
Sterile swing stopper sample bottle (50 mL) or other suitable flask
Sterile swabs tubes or sterile test tubes with screw caps or porous stoppers
Sterile swabs
Incubator for anaerobic samples

Application
Handle the samples under sterile conditions to avoid secondary contamination.

Due to its sensitivity to high temperatures please avoid heating the NBB®-B. Use sterile test tubes with screw caps or porous stoppers (e.g. plastic-foam plugs) or sterile swing stopper sample bottles. While filling the bottles directly from the original NBB®-B flask take care to work close to a Bunsen burner flame to avoid a possible contamination.

For yeast analysis place 1 to 4 mL of the sample (depending on the yeast concentration) in a 50 mL swing stopper sample bottle or other suitable flask. Add 20-30 mL of NBB®-B.

Yeast samples can also be examined very handy with sterile swabs (preferably with screw cap). Collect your yeast sample (approx. 0.5 mL) directly from several points of yeast container and fill the tube to about 70% with NBB®-B (Fig. 1). The screw should not be tightened firmly during incubation to allow CO_2 to escape from the tube. Proceed in the same way with sediments of unfiltered beer.

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this version replaces all previous versions.

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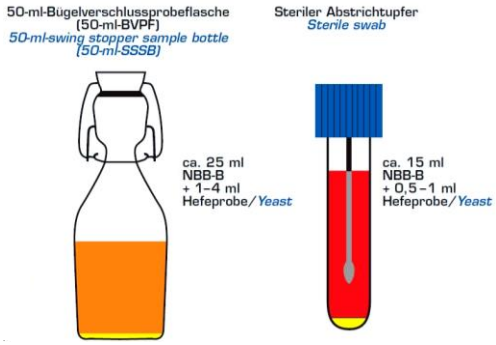

Figure 1: Preparation of samples

As an enrichment medium take a sterile swing stopper sample bottle (50 mL) or other suitable bottle and mix 2.5-10 mL NBB®-B (depending on the contamination) with the filtered beer sample to the bottle edge.

Incubation

Place the test sample into an incubator between 25-28°C/77-82°F and incubate for 5 days. Samples not containing yeast cells should be incubated under anaerobic conditions. Incubate the enrichment probe for 2 days for further use with NBB®-A; NBB®-B or molecular biological methods (VIR, PCR).

Evaluation

If the contaminations are high enough and if the organisms are typical beer spoilers, the colour of the indicator in the samples will turn from red to yellow.
Since the speed of the microbial growth depends on several factors (initial cell count, type, physiological condition and origin of the germs, degree of adaptation to beer), an incubation time of one day may be sufficient, in case of high contaminations, to obtain a result. Mostly several days are needed in case of trace contaminations or of very slow growing strains (e.g. *Lactobacillus lindneri*).Observe the growth-pattern during incubation time to obtain clear statements about the condition of your sample. Evaluate your samples after 5 days of incubation for a final comparability of your results. In case of trace contaminations or older, autolyzing yeast cells from the tank bottom, the indicator colour may not be evident enough to get a clear result. In this case the samples have to be examined under the microscope.

3. Storage and Packaging Information

Packaging and Content

unit	Cardboard Box (9x250 mL bottles)
unit size (Box)	approx. 22 cm x 22 cm x 18 cm / 8.7 in x 8.7 in x 7.1 in
unit gross weight (Box)	approx. 4.1 kg/ 9 lbs.

Storage

Store at 4-8°C/40-46°F according to product specification.
Store under dry and dark conditions. Do not freeze product.

User manual

Waste Disposal
No dangerous good.
No hazardous material.
Please consider your local waste regulations.
Not inoculated broth can be disposed with normal laboratory waste.
Inoculated and incubated samples are to be sterilized before disposal at a temperature of 121°C /250°F for 20 min.

Warnings
Do not cook or freeze the product.

4. Related products

Sample type	Product	Format	Method	Item no.	Packaging	Incubation			Analysis
						T [°C]	t [d]	Condition	
Yeast samples Selected, harvested and brewing yeast Yeast sediments	**NBB®-B**	Broth in bottle	0.5-1 ml of sample + 10-20 ml of NBB®-B	2.04710.782	9 x 250 ml (glass bottle)	28°C	3-5	aerobic	qualitative
		Broth in tubes		2.04723.646	20 x 10 ml (tube)				
Yeast-cloudy beers Green beer Unfiltrate Wheat beer	**NBB®-C**	Concentrated broth	95 % of sample + 5 % of NBB®-C	2.04711.782	9 x 250 ml (glass bottle)	28°C	7-12	anaerobic	qualitative
Clear beers Membrane filtered samples	**NBB®-A**	Agar	Filtration of 50-200 ml of sample	2.04709.782	9 x 250 ml (glass bottle)	28°C	5-7	anaerobic	quantitative
Water, rinsing water Membrane filtered samples	**NBB®-A**	Agar	Filtration of 50-200 ml of sample	2.04709.782	9 x 250 ml (glass bottle)	28°C	5-7	anaerobic	quantitative
Environmental air Sampling of airborne microorganism on agar plates	**NBB®-A**	Agar	Direct sampling	2.04709.782	9 x 250 ml (glass bottle)	28°C	5-7	anaerobic	quantitative
Surfaces in filling plants Hygiene monitoring using swabs	**NBB®-B-AM**	Broth	1 swab in 10 ml of NBB®-B-AM	2.04706.782	9 x 250 ml (glass bottle)	28°C	3	aerobic	qualitative
	NBB®-P	Powder	For the in-house production of NBB®-A and NBB®-B, using beer from own production.	2.04716.462	300 g (bag)				
Laboratory accessories	**Smear swabs, without tube**			2.04725.444	100 pc. (bag)				