NCM1017/700006972-5

Product Instructions

- EN One Plate Yeast and Mould (OP-YM)
- FR One Plate Yeast and Mould (OP-YM)
- DE One Plate Yeast and Mould (OP-YM)
- ES One Plate Yeast and Mould (OP-YM)
- PT One Plate Yeast and Mould (OP-YM)
- JA One Plate Yeast and Mould (OP-YM)
- ZH One Plate Yeast and Mould (OP-YM)
- KO One Plate Yeast and Mould (OP-YM)

Product Instructions

One Plate Yeast and Mould (OP-YM)

Intended Use

One Plate Yeast and Mould (OP-YM) offers a rapid method for the enumeration of yeast and mould using traditional culture methodology regardless of the water activity (a_w).

Product Summary and Explanation

Fungi are divided into two main groups: yeasts and moulds. Yeasts are single-cell organisms, whereas moulds grow as multicellular filaments containing multiple identical nuclei. Fungi are a leading cause of spoilage in foodstuffs and potentially produce harmful toxins. Both yeasts and moulds cause various degrees of deterioration and decomposition of foods. They can contaminate any type of food and invade crops such as grains, nuts, beans, and fruits in fields at any time, from the field to the store. Processed foods and food mixtures can also be affected.

One Plate Yeast and Mould is a selective and differential agar for the enumeration of yeasts and moulds in a broad range of foods. It can permit a quantitative result for yeast and mould in a minimum of 54 hours for all food products (all water activities), using only one plate versus 2 or more as described in ISO 21527-1, ISO 21527-2, and ISO 7218.

The product is composed of an agar base, providing all the required growth agents to permit rapid enumeration of yeast and mould from any sample type regardless of water activity. The selective agents prevent bacterial growth from interfering with fungal count. Glycerol is added to the base pre-sterilisation and is used regardless of sample type. A supplement is then added to the molten agar post-sterilisation, which primarily produces diagnostic colouration of yeast species.

Intended User

The method is designed for use by qualified personnel with appropriate training.

Product Codes

NOTE: Please refer to ISO 6887 parts 1–5 for suitable sample specific diluents.

Product Name	Format	Pack Size	Product Code	
One Plate Yeast and Mould Agar	DCM	500 g	NCM1017A	700006972
		5 kg	NCM1017B	700006973
		10 kg	NCM1017C	700006974
		25 kg	NCM1017D	700006975
One Plate Yeast and Mould Diagnostic Supplement	Liquid-Ready Supplement	1 x 100 mL bottle (for up to 50 L media)	NCM4088-50	700006976
One Plate Yeast and Mould Agar	Prepared Agar Plates*	20 x 90 mm Agar Plates	On Request	
		100 x 90 mm Agar Plates	On Request	
One Plate Yeast and Mould Agar	Ready to Re-melt Agar**	1 x 250 mL bottle	On Request	

*Prepared Agar Plates do not require supplementation.

**Ready to Re-melt Agar should be melted in a steaming water bath (refer to ISO 7218 for further guidance). Media should be supplemented after tempering.

Typical Composition

Formulation may be adjusted and/or supplemented as required to meet performance specifications.

Peptones & Extracts	5.25 g/L
Growth Enhancers	12.0 g/L
Buffer Mix	4.0 g/L
Selective Mix	0.1 g/L
Agar	11.0 g/L

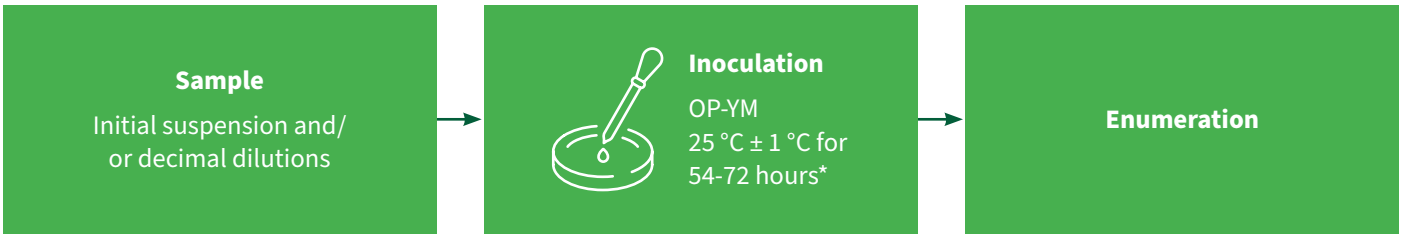
pH of the prepared media at 25 °C: pH 5.6 ± 0.2.

Preparation

Suspend 32.35 grams of the medium in 960 mL of purified water and add 40 grams of glycerol (CAS 56-81-5.) Agitate frequently to completely dissolve the medium. Autoclave at 121 °C for 15 minutes. Cool to 45–50 °C. Aseptically add 2.0 mL of the One Plate Yeast and Mould diagnostic supplement (NCM4088-50), swirl to mix, and pour into Petri dishes.

NOTE: The One Plate Yeast and Mould diagnostic supplement does not contribute any growth requirements. If the supplement is not added, yeast colonies present as their natural straw/white colouration.

OP-YM Flow Diagram



* Plates can be incubated for up to 72 hours for convenience.

Sample Preparation

Follow the specifications of ISO 6887 or the specific international standard appropriate to the product concerned for the initial suspension and dilutions.

Surface Inoculation

1. Transfer 0.1 mL of the suspension, or any serial dilutions, onto the surface of **ONE** single plate of prepared or pre-poured OP-YM agar plate.
2. Spread the inoculum on the surface with the aid of a sterile spreader.

NOTES: Automatic spiral plating deposition techniques can be used for surface inoculation. Decimal dilutions could be required if typical contamination range is unknown. To estimate small numbers, spread 1 mL of the primary dilution over the surface of 3 prepared plates as described in ISO 7218.

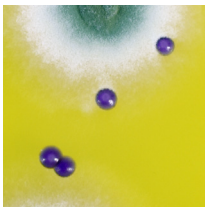
Pour Plate Inoculation

1. Transfer 1 mL of the sample if liquid, or 1 mL of the suspension in the case of other products, or any serial dilutions, into **ONE** empty, sterile Petri dish.
2. Pour approximately 15–20 mL of molten OP-YM into the plate. Homogenize well by swirling, and let solidify on a cool surface.

NOTE: Overlays are not required.

Incubation

Invert the prepared plate and incubate at 25 ± 1 °C for 54–72 hours.



Interpretation

Yeast interacts with the substrate in the diagnostic supplement to produce lilac to purple colonies. Mould will present their natural pigmentation but may acquire colour from the substrate in later stages of growth. Yeast produces matte or shiny colonies, whereas mould produces flat or fluffy spreading propagules or colonies, often with colored fruiting or sporing structures. Using this morphological difference, yeast and mould can be counted separately if desired.

Counting Colony-forming Units

Select the plate containing 10–150 colonies/propagules/germs for accurate enumeration. Refer to the ISO 7218 when the results are outside the limits. Apply the dilution factors, eliminating the need for two successive dilutions or duplicates.

Different yeast species produce different levels of colouration from the substrate, but a combination of morphology and colour observation will allow for total yeast count. Mould present various different colors but can be better differentiated by morphology. To enumerate total yeast and mould, count all colonies regardless of colour or morphology.

Spreading colonies are considered single colonies. If less than one quarter of the dish is overgrown by spreading, count the colonies on the unaffected part and extrapolate for the theoretical total count of the dish.

Confirmation is not required for fungal enumerations.

Dilute to Specifications Results

Dilutions are made according to the specification, and a calculated amount of the appropriate dilution is added to the plate.

Examples of specification dilutions:

Dilution	Inoculation	Specifications
1/10	1 mL pour plate	Negative result <10 CFU/mL Positive result ≥ 10 CFU/mL
1/10	0.1 mL surface or pour plate	Negative result <100 CFU/mL Positive result ≥ 100 CFU/mL
1/10	0.01 mL surface (loop) streak plate	Negative result <1000 CFU/mL Positive result ≥ 1000 CFU/mL
1/100	1 mL pour plate	Negative result <100 CFU/mL Positive result ≥ 100 CFU/mL
1/100	0.1 mL surface or pour plate	Negative result <1000 CFU/mL Positive result ≥ 1000 CFU/mL
1/1000	1 mL pour plate	Negative result <1000 CFU/mL Positive result ≥ 1000 CFU/mL

Quality Control

Appearance of dehydrated media: Beige, homogeneous, free-flowing powder
Appearance of prepared media (after supplement addition): Fluorescent to yellow gel

Typical cultural response when incubated aerobically at 25 ± 1 °C and examined for growth at 54–72 hours:

Microorganism	WDCM	Approx. Inoculum (CFU)	Expected Results
Saccharomyces cerevisiae	00058	50–200	>50% Recovery, Iliac/purple
Candida albicans	00054	50–200	>50% Recovery, Iliac/purple
Aspergillus brasiliensis	00053	50–300	>50% Recovery
Eurotium rubrum	00184	50–300	>50% Recovery
Escherichia coli	00012	$>10^4$	Complete Inhibition
Bacillus subtilis	00003	$>10^4$	Complete Inhibition

Precautions and Limitations of the Method

1. Use good microbiology laboratory practices as per ISO 7218, except mandatory duplicate plating for enumeration, as the One Plate method requires only one Petri dish.
2. Whilst yeast and mould can be differentiated on morphology, there are some intermediate forms, thus enhanced examination may be required using a binocular magnifier or microscope.
3. Colony counts in excess of 150 CFU per plate can lead to poorer definition of the diagnostic reactions; it is advised that a higher dilution be used to accurately enumerate higher levels of contamination.
4. Both the limit of detection and quantification are dictated by the subculture volume.
5. An inoculation of 0.1 mL (spread plate) of a 1/10 dilution has a limit of detection of 100 CFU/g and permits the user to quantify (enumerate) > 1000 CFU/g.
6. An inoculation of 1.0 mL (pour plate) of a 1/10 dilution has a limit of detection of 10 CFU/g and permits the user to quantify (enumerate) > 100 CFU/g.
7. If a plate count results in less than 10 colonies counted, an estimated number should be expressed, e.g., report >10 CFU/g or >100 CFU/g (depending on the inoculation volume).
8. Enumeration, especially of mould, can be imprecise because of the mix of mycelium and asexual or sexual spores. Final count will depend on degree of fragmentation of mycelium, but risk can be minimized by proper sample homogenization.
9. Care should taken when handling Petri dishes with mould growth, as spores can contaminate the environment.

Verification

Method verification should be done following the protocols described in ISO 16140:3:2021. The user laboratory should follow experimental design and acceptance criteria as described in chapter 5, ‘Quantitative methods — Technical protocol for verification’.

Validations

MicroVal (ISO 16140-2:2016)

One Plate Yeast and Mould has been certified by MicroVal as an alternative to the reference ISO 21527-1:2008 Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0,95 and ISO 21527-2:2008 horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0,95, according to the reference standard ISO 16140-2:2016 with the scope of enumeration of yeast and moulds in a broad range of foods. Refer to the MicroVal certificate for more information.

Safety

Refer to the relevant product safety data sheet (SDS). Wear protective gloves/protective clothing/eye protection/face protection, and suitable respiratory protection. The dehydrated agar base contains chloramphenicol, which is a carcinogen and a serious health hazard in its powdered/dust format.

Storage

Store dehydrated culture media at 2–30 °C away from direct sunlight. Once it is opened and recapped, place the container in a low-humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Prepared media and/or supplements should be stored at 2–8 °C. Do not freeze. Supplement may be stored at room temperature for up to 5 days.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free-flowing, or if appearance has changed from the original colour. Expiry applies to medium in its intact container when stored as directed.

Disposal

Cultures should be disposed of appropriately as biohazard waste. The preferred method of treatment for biohazard waste is autoclaving. Items that cannot be autoclaved may be disinfected with bleach solution. Consult with the safety advisor for your facility for detailed instructions.

Customer Service

Neogen Customer Services and Technical Services can be reached by using the following contact information:

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+44 (0) 1292 525600
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Training on this product, and all Neogen test kits, is available.

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