Product Information

MetaPolyzyme, DNA free

Suitable for Microbiome research

MAC4LDF

Synonym: Multilytic Enzyme Mix

Storage Temperature –20 °C

Product Description

Metagenomics investigates all DNA that has been isolated directly from given single samples, such as environmental samples or biological organisms.[1,2] Metagenomics allows for the investigation of microbes that exist in extreme environments, and which have been historically difficult to isolate, culture, and study.[3] Metagenomics has revealed the existence of novel microbial species.[4] Applications of metagenomics work include public health data analysis,[5,6] discovery of novel proteins, enzymes and natural products,[7,8] environmental studies,[9,10] and agricultural investigations.[11,12]

Microbes are difficult to disrupt because the cell walls may form capsules or resistant spores. DNA can be extracted by using lysing enzymes such as lyticase, chitinase, zymolase, and gluculase to induce partial spheroplast formation. Spheroplasts are subsequently lysed to release DNA.

MetaPolyzyme products (Cat. Nos. MAC4L, MAC4LDF) are based on a multi-lytic enzyme mixture, originally developed by Scott Tighe, for use in microbiome and DNA extraction efficiency studies, and formulated for effective lysis of microbiome samples from extreme environments. MetaPolyzyme was originally evaluated and developed in consultation and collaboration with the Association of Biomolecular Resource Facilities (ABRF) Metagenomics and Microbiome Research Group (MMRG; formerly the Metagenomics Research Group, MGRG).[13-16]

Studies of microbial communities have been enhanced by the use of culture-independent analytical techniques such as 16S rRNA gene sequencing and metagenomics. DNA contamination during sample preparation is a major problem of sequence-based approaches. Extraction reagents free of DNA contaminants are thus essential.

MetaPolyzyme, DNA free was developed to address the need for DNA-free reagents, to minimize microbial DNA contamination from reagents. This product undergoes strict quality control testing to ensure the absence of detectable levels of contaminating microbial DNA using 35 cycles PCR amplification of 16S and 18S rDNA using universal primer sets.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagent

The enzymes in MetaPolyzyme, DNA free are:

- Mutanolysin
- Achromopeptidase
- Lyticase
- Chitinase
- Lysostaphin
- Lysozyme

All the enzymes are individually tested for the absence of contaminating DNA using 16S and 18S PCR amplification.

Mutanolysin (from *Streptomyces globisporus*)

Mutanolysin is a muralytic enzyme (muramidase) that cleaves the β-*N*-acetylmuramyl-(1→4)-*N*-acetylglucosamine linkage of the bacterial cell wall peptidoglycan-polysaccharide, particularly the β(1→4) bond in MurNAc-GlcNAc.[17] Mutanolysin particularly acts on many Gram-positive bacteria, where the enzyme's carboxy-terminal moieties participate in the recognition and binding of unique cell wall structures.

Achromopeptidase

Achromopeptidase (known also as β-lytic protease[18]) has potent bacteriolytic activity on many Gram-positive aerobic bacteria[19] with high lytic activity, against bacterial strains with the A1α chemotype (such as *Aerococcus viridans*), and the A3α chemotype (such as *Staphylococcus epidermidis*) for cell wall peptidoglycan structures. The enzyme has been reported to have particular recognition for Gly-X sites in peptide sequences, and for Gly-Gly and D-Ala-X sites in peptidoglycans.[20]

Lyticase (from *Arthrobacter luteus*)

Lyticase is useful in digestion of linear glucose polymers with β(1→3) linkages, of yeast glycan coats and for spheroplast formation, and of the cell wall of active yeast cells.

Chitinase (from *Streptomyces griseus*)

Chitinase degrades chitin by enzymatic hydrolysis to N-acetyl-D-glucosamine. Degradation occurs via two consecutive enzyme reactions:

- Chitodextrinase-chitinase, a poly(1,4-β-[2-acetamido-2-deoxy-D-glucoside])-glycanohydrolase, removes chitobiose units from chitin.
- N-acetylglucosaminidase-chitobiase cleaves the disaccharide to its monomer subunits, N-acetyl-D-glucosamine (NAGA).

Lysostaphin (from *Staphylococcus staphylolyticus*)

Lysostaphin is a lytic enzyme with activity against *Staphylococcus* species, including *S. aureus*. Lysostaphin has hexosaminidase, amidase, and endopeptidase activities. It cleaves polyglycine crosslinks in the cellular wall of *Staphylococcus* species, which leads to cell lysis.[21,22]

Lysozyme (from chicken egg white)

Lysozyme hydrolyzes β(1→4) linkages between *N*-acetylmuraminic acid and *N*-acetyl-D-glucosamine residues in peptidoglycan, and between *N*-acetyl-D-glucosamine residues in chitodextrin. Lysozyme lyses the peptidoglycan cell wall of Gram-positive bacteria.[23]

Storage/Stability

This product ships at cooler temperature conditions. Long-term storage at –20 °C is recommended.

Reconstituted solutions of MetaPolyzyme, DNA free may be stored at –20 °C, but long-term solution stability has not been examined.

Preparation Instructions

Because of the great diversity of samples for metagenomics studies, it will be necessary for each researcher to work out particular conditions for optimal sample preparation and treatment.

It is recommended to reconstitute MetaPolyzyme, DNA free in sterile PBS buffer, pH 7.5 (no EDTA, calcium or magnesium present in solution). The following is a sample procedure, to be scaled appropriately:

1. Add 100 µL of sterile PBS (pH 7.5) to 1 vial of MetaPolyzyme, DNA free.
    1.1. Resuspend by gentle agitation or pipetting.
    1.2. Set solution aside at 2-8 °C until Step 7.
2. Thoroughly suspend sample in sterile PBS (pH 7.5).
3. Add 200 µL of sample in PBS to a 2 mL polypropylene microcentrifuge tube.
4. Optional pellet wash:
    4.1. To sample tube, add 1 mL of PBS (pH 7.5).
    4.2. Vortex, centrifuge and remove supernatant.
    4.3. Repeat pellet wash two more times if needed.
5. Resuspend pelleted sample in 150 µL of PBS (pH 7.5). Vortex thoroughly.
6. Optional: if solution will sit for more than 4 hours, sodium azide may be added to 0.02%.
7. Add 20 µL (*) of MetaPolyzyme, DNA free to sample solution.
8. Incubate at 35 °C for 2-24 hours.
9. Continue with standard DNA extraction protocol.

(*) The optimal volume and concentration of MetaPolyzyme, DNA free may vary in different experiments.

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