Technical Bulletin

MetaPolyzyme Multilytic Enzyme Mix

MAC4L
Storage Temperature –20 °C

Product Description

Metagenomics is a rapidly expanding field of basic and applied research which looks at all DNA that has been isolated directly from given single samples (e.g. environmental samples, 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 metagenomic studies 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]

MetaPolyzyme is a mix of enzymes which have proven useful in metagenomic studies of microbiomes of extreme and unique environments.[13-16] The product is intended for digestion of the microbes for evaluation by whole genome shotgun sequencing for metagenomics and metatranscriptomic approaches.

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. The original version of this enzyme blend was formulated with the intent to facilitate more effective lysis of spheroplasts (or protoplasts).[16]

This product was 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] Several publications have cited use of this product.[17-19]

The enzymes in MetaPolyzyme are:

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

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.[20] 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[21]) has potent bacteriolytic activity on many Gram-positive aerobic bacteria[22] with high lytic activity, against bacterial strains with the A1α chemotype (e.g., Aerococcus viridans), and the A3α chemotype (e.g., 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.[23]

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-acetylD-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.[24,25]

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-acetylD-glucosamine residues in chitodextrin. Lysozyme lyses the peptidoglycan cell wall of Gram-positive bacteria.[26]

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.

Preparation Instructions

Dissolve the contents of MetaPolyzyme by adding 1 mL of PBS, pH 7.5, to make a 5 mg/mL solution. There should be no EDTA, Calcium or Magnesium present in solution.

Storage/Stability

This product ships at cooler temperature conditions ('wet ice'). Long-term storage at –20 °C is recommended. Reconstituted solutions of MetaPolyzyme may be stored at –20 °C, but long-term solution stability has not been examined.

Procedure

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. The following items should be noted before sample preparation:

- PBS buffer used for MetaPolyzyme and sample preparation should be at pH 7.5 and must not have EDTA, calcium or magnesium present in the solution.
- Sample with MetaPolyzyme must not be combined with Proteinase K.
- Sample with MetaPolyzyme must not be combined with lysis buffer or detergent.
- Sample suspected of containing interfering conditions mentioned above may be washed in 2X PBS before resuspending in PBS and adding MetaPolyzyme.
- MetaPolyzyme is also compatible with additional Lysozyme.
- It is recommended to also prepare 2 controls of PBS solution: one with MetaPolyzyme and one without MetaPolyzyme, as needed.
- The procedure below describes sample preparation in 2 mL microcentrifuge tubes. This procedure can be used as a guide to scale appropriately (ex: 96 well plate, swab samples).

1. Add 1 mL of PBS, pH 7.5 (without EDTA, calcium, magnesium) to MetaPolyzyme to create 5 mg/mL solution, set aside until ready for step 6.

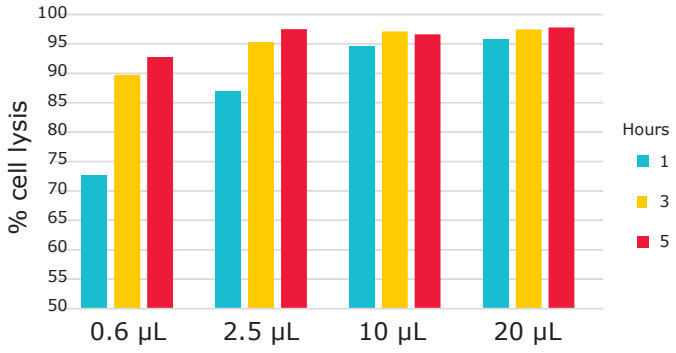
   Recommended: Aliquot MetaPolyzyme solution into desired aliquots for future use (example: 10 vials of 100 uL) Aliquots can be stored in -20 °C, but long-term stability of solution has not been examined. If solution will sit for more than 4 hours, add sodium azide to 0.02%.

2. Thoroughly suspend sample in PBS, pH 7.5.
3. Add 200 µL of sample in PBS to 2 mL polypropylene microcentrifuge tube.
4. Optional pellet wash: To sample tube, add 1 mL of PBS, pH 7.5 Vortex, centrifuge and remove supernatant. Repeat step 4 two more times.
5. Resuspend pelleted sample in 150 µL of PBS, pH 7.5 and vortex thoroughly.
6. Add *5-20 µL of MetaPolyzyme to sample solution.
7. Incubate at 35 °C for 2-24 hours.
8. Follow your standard DNA extraction protocol.

* Application data collected by the ABRF:

MetaPolyzyme Treatment

200 µL sample of pure bacteria in PBS 7.5, 35 °C



Volume of 5 mg/mL MetaPolyzyme added to 200 µL sample

Each 200 uL of pure bacteria sample was treated with 0.6 µL, 2.5 µL, 10 µL or 20 µL. The MetaPolyzyme + sample was incubated for 1, 3, and 5 hours at 35 °C. An OD reading of each sample per timepoint was recorded, as well as a sample with no MetaPolyzyme and a control of PBS only. A decrease in OD indicates an increase in cell lysis, so data is expressed as a % cell lysis, compared to the sample with no MetaPolyzyme (which would have 0% lysis). The treatment data shows that concentration and incubation time affect the % cell lysis of pure bacteria samples. Therefore, it is recommended that each user works out specific conditions for optimal sample preparation and treatment.

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