Product Information

MetaPolyzyme with RNase Inhibitor

Lytic enzyme mixture for RNA extraction

MAC4LRI

Synonyms: Multilytic Enzyme Mix suitable for RNA isolation

Storage Temperature −20 °C

Product Description

Bacterial Metagenomics and Transcriptomics are rapidly expanding fields of basic and applied biological research. Novel approaches make it possible to directly analyze DNA as well as the corresponding RNA isolated from a given single sample (for example, environmental samples, biological organisms)[1,2]. Historically, Metagenomics explored bacterial communities that were difficult to isolate, and study,[3] describing the structure of the microbial communities and revealing the existence of new microbial species.[4] Nevertheless, in recent years there has been a growing interest in understanding not only the composition of the bacterial community but also the underlying functionality, as manifested by their intricate transcriptome.[5]

As technology progresses and novel methods are used to research the Metagenome of a large variety of microbiome samples, new challenges arise, and new solutions are needed. A common difficulty among microbiome scientists is to extract nucleic acids from difficult to lyse microbes that form capsules or highly resistant spores. To address this challenge, protocols that integrate lysing enzymes can be incorporated into the nucleic acid extraction workflow. Nucleic acids can be extracted by using various lytic enzymes to induce partial spheroplast formation. Spheroplasts are subsequently lysed to release nucleic acids. As such, data has shown that adding a pre-digestion step to nucleic acid extraction with lytic enzymes mixtures such as MetaPolyzyme (MAC4L) results in increased recovery of both low and high MW DNA on many sample types that are suitable for long-read sequencing applications.[6,10]

Isolating RNA from microbiome samples poses additional challenges. RNA molecules are highly labile and are prone to degradation by contaminating RNases. As such, to adapt the MetaPolyzyme for RNA isolation, a broad range RNase inhibitor has been incorporated to help prevent RNA degradation by various RNases. The new product, MAC4LRI, includes all 6 enzymes from MetaPolyzyme to ensure maximal lysis of microorganisms while protecting the sample RNA from degradation. While it is not possible to inhibit all RNases from a specific microbial environment, we have incorporated a proprietary RNase Inhibitor to help inhibit as many RNases as possible.

The MetaPolyzyme product family includes MAC4L, MAC4LDF (DNA-Free), SAE0200 (MycoPolyzme, for yeast and fungal lysis, DNA-Free) and MAC4LRI (RNase Inhibitor) and are based on a multi-lytic enzyme mixture formulated for effective lysis of microbiome samples from any sample type. Originally, MetaPolyZyme was developed by Scott Tighe for use on samples from extreme environments and general applications to increase DNA extraction efficiency for any method. To date, these products have been further evaluated and continuously 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),[7-10] Several publications have cited use of MetaPolyzyme, suggesting various advantages to the usage of MetaPolyzyme for enhancing bacterial lysis.[11-13]

The components in MetaPolyzyme with RNase inhibitor (MAC4LRI) are:

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.[14] 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 (also known as β-lytic protease[15]) has potent bacteriolytic activity on many Gram-positive aerobic bacteria[16] with high lytic activity, against bacterial strains with the A1α chemotype (for example, *Aerococcus viridans*), and the A3α chemotype (for example, *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.[17]

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.[18,19]

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.[20]

RNase inhibitor

A highly potent, broad range RNase inhibitor that inhibits the major contaminating eukaryotic RNases, as well as some common bacterial and fungal RNases.

Storage/Stability

Recommended long-term storage before reconstitution is at −20 °C.

Reconstituted solutions of MetaPolyzyme with RNase inhibitor may be stored at −20 °C, however long-term solution stability has not been examined.

Preparation Instructions

Due of the great diversity of samples extracted in microbiome research, it is necessary for each researcher to optimize incubation time and concentration to obtain maximal performance.

It is recommended to reconstitute in sterile DNA-free PBS (MBD0058) buffer, pH 7.5 (no EDTA, calcium or magnesium present in solution). Below is an example procedure, to be scaled appropriately:

1. Add 100 µL of sterile, Nuclease and RNase free H_2O (such as W4502 or equivalent), to 1 vial of MAC4LRI. Resuspend by gentle agitation or pipetting. Set solution aside at 2-8 °C until step 6.
2. Thoroughly suspend approximately 50-100 mg of sample in equal volume (or more, if needed) of sterile PBS pH 7.5. Mix by Vortex.
3. Add up to 200 µL of sample in PBS to a 2 mL polypropylene microcentrifuge tube. If later steps include bead beating, use a tube containing beads.
4. Optional pellet wash: To the sample tube from 3., add 1 mL of PBS, pH 7.5. Vortex, centrifuge and remove supernatant. Repeat step 4 two more times if needed.
5. Resuspend pelleted sample in 100 µL of PBS, pH 7.5 and gently vortex.

Note: If solution is expected to incubate for extended periods, the addition of Sodium-Azide to a final concentration of 0.02% is recommended to prevent bacterial regrowth. However, this is not typically required for the short incubation of an RNA sample digestion.

6. Add 5-10 µL of MetaPolyzyme with RNase inhibitor to sample solution.

   Note: The optimal volume and concentration of MAC4LRI may vary in different experiments.

7. Incubate at 35 °C for 15-30 minutes.

   Note: For samples that have very high levels of RNases, it is recommended to add RNA extraction buffer immediately and MAC4LRI is not recommended. In this scenario without the use of MAC4LRI, there will be less access to RNA from hard to lyse microorganisms.

8. Continue with standard RNA extraction protocol.

Note: MetaPolyZyme enzymes are active in PBS solution. The presence of EDTA or extraction buffer such as a chaotropic salt (RLT/Guanidinium/Trizol/ SDS) will deactivate the enzyme activity immediately.

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