Apyrase

Applications:
- Highly efficient degradation of ATP to AMP.
- Removal of deoxynucleotides in DNA pyrosequencing between cycles (3).
- Conversion of 5’ triphosphorylated RNA to ligatable monophosphorylated form that can be used for 5’ RNA adaptor ligation.
- Conversion of 5’ triphosphorylated RNA to 5’ exonuclease XRN-1 (NEB #M0338) sensitive monophosphorylated RNA.

Source: Isolated from a strain of E. coli that carries the coding sequence for potato S. tuberosum apyrase (4).

Supplied in: 50 mM NaCl, 20 mM MES (pH 6.5 @ 25°C), 0.1 mM CaCl₂, 1 mM DTT, 0.1% Tween-20 and 50% glycerol.

Reagents Supplied with Enzyme:
10X Apyrase Reaction Buffer

Reaction Conditions: 1X Apyrase Reaction Buffer Incubate at 30°C.

1X Apyrase Reaction Buffer:
- 20 mM MES (pH 6.5 @ 25°C)
- 50 mM NaCl
- 5 mM CaCl₂
- 1 mM DTT
- 0.05% Tween-20

Unit Definition: One unit is defined as the amount of enzyme that catalyses the release of 1 μmol of inorganic phosphate from ATP (1 mM, NEB #P0756) in a 1X Apyrase Reaction Buffer in 1 minute at 30°C in a total reaction of 50 μl.

Specific Activity: 3,000 units/ml

Molecular Weight: 47 kDa

Heat Inactivation: 65°C for 20 minutes

Notes:
- Apyrase has a higher ratio of activity for ATP:ADP (14:1).
- Apyrase is a calcium-activated enzyme. It is approximately 50% active when Mg²⁺ substitutes Ca²⁺ in Apyrase Reaction Buffer.

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- As a metal-dependent enzyme Apyrase can be inhibited by EGTA and EDTA.
- The activity of Apyrase is approximately 30% in NEBuffers 1.1, 2.1, 3.1 and CutSmart® Buffer.
- Apyrase does not remove 5’ caps from eukaryotic mRNA.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Phosphatase Activity (PNPP Assay): Incubation of a 50 μl reaction in 1X Apyrase Reaction Buffer containing 10 mM p-Nitrophenyl Phosphate (PNPP) and a minimum of 5 units of Apyrase for 4 hours at 37°C results in < 0.1% substrate dephosphorylation as determined by spectrophotometric analysis.

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**RNase Activity:** Incubation of a 10 µl reaction in 1X NEBuffer 4 containing a minimum of 1 µl of Apyrase and 40 ng of F-300 RNA transcript incubated for 16 hours at 37°C results in < 10% substrate degradation as determined by gel electrophoresis using fluorescent detection.

**Exonuclease Activity:** Incubation of a 50 µl reaction in 1X Apyrase Reaction Buffer containing a minimum of 5 units of Apyrase with 1 µg of a mixture of single and double-stranded [3H] E. coli DNA for 4 hours at 30°C releases < 0.1% of the total radioactivity.

**Endonuclease Activity:** Incubation of a 50 µl reaction in 1X Apyrase Reaction Buffer containing a minimum of 5 units of Apyrase with 1 µg of supercoiled φX174 DNA for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis using ethidium bromide detection.

**Single Stranded DNase Activity (FAM-Labeled Oligo):** A 50 µl reaction in CutSmart Reaction Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 2.5 units of Apyrase incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

**DNase Activity (Labeled Oligo, 3’ extension):** A 50 µl reaction in CutSmart Reaction Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3’ extension and a minimum of 2.5 units of Apyrase incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

**DNase Activity (Labeled Oligo, 5’ extension):** A 50 µl reaction in CutSmart Reaction Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5’ extension and a minimum of 2.5 units of Apyrase incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

**DNase Activity (Labeled Oligo, blunt end):** A 50 µl reaction in CutSmart Reaction Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 2.5 units of Apyrase incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

**DNase Activity (Labeled Oligo, 3´ extension):** A 50 µl reaction in CutSmart Reaction Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3’ extension and a minimum of 2.5 units of Apyrase incubated for 16 hours at 37°C yields < 5% degradation as determined by capillary electrophoresis.

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