Exonuclease V (RecBCD)

Source: An *E. coli* strain containing plasmids for expressing the three subunits of *E. coli* Exonuclease V: RecB, RecC and RecD.

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme: 10X NEBuffer 4, 10 mM ATP

Reaction Conditions: 1X NEBuffer 4 supplemented with 1 mM ATP. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37°C in a total reaction volume of 50 µl.

Unit Assay Conditions: 1X NEBuffer 4, 1 mM ATP with 0.15 mM sonicated duplex [H]-DNA.

Heat Inactivation: 70°C for 30 minutes.

Quality Control Assays

Endonuclease Activity I: Incubation of a 50 µl reaction containing 100 units of Exonuclease V with 1 µg of φX174 RF I DNA in NEBuffer 4 and 1 mM ATP for 4 hours at 37°C resulted in < 10% loss in φX174 RF I DNA as determined by agarose gel electrophoresis.

Endonuclease Activity II: Incubation of a 50 µl reaction containing 50 units of Exonuclease V with 1 µg of φX174 RF II DNA in NEBuffer 4 and 1 mM ATP for 4 hours at 37°C resulted in < 10% loss in φX174 RF II DNA as determined by agarose gel electrophoresis.

RNase Assay: Incubation of 10 units of enzyme with 40 ng of 300 bases Fam-labeled RNA at 37°C for 3 hours in a 50 µl reaction buffer results in < 10% of RNA degradation as analyzed from polyacrylamide gel.

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

A Typical Exonuclease V Reaction:
1 µl sample DNA (~ 1 µg)
3 µl NEBuffer 4 (10X)
3 µl 10 mM ATP
y µl H2O (up to final volume of 30 µl)
1 µl Exonuclease V (10 units)
1. Incubate at 37°C for 30 minutes.
2. To stop reaction add EDTA to 11 mM.
3. Heat Inactivation 70°C for 30 minutes.
4. Clean-up treated samples by column purification and/or ethanol precipitation.

Note: Estimate amount of DNA to be removed by agarose gel electrophoresis or OD260. If > 1 µg scale up all reaction components proportionately.

---

Exonuclease V (RecBCD)

Source: An *E. coli* strain containing plasmids for expressing the three subunits of *E. coli* Exonuclease V: RecB, RecC and RecD.

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

Reagents Supplied with Enzyme: 10X NEBuffer 4, 10 mM ATP

Reaction Conditions: 1X NEBuffer 4 supplemented with 1 mM ATP. Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37°C in a total reaction volume of 50 µl.

Unit Assay Conditions: 1X NEBuffer 4, 1 mM ATP with 0.15 mM sonicated duplex [H]-DNA.

Heat Inactivation: 70°C for 30 minutes.

Quality Control Assays

Endonuclease Activity I: Incubation of a 50 µl reaction containing 100 units of Exonuclease V with 1 µg of φX174 RF I DNA in NEBuffer 4 and 1 mM ATP for 4 hours at 37°C resulted in < 10% loss in φX174 RF I DNA as determined by agarose gel electrophoresis.

Endonuclease Activity II: Incubation of a 50 µl reaction containing 50 units of Exonuclease V with 1 µg of φX174 RF II DNA in NEBuffer 4 and 1 mM ATP for 4 hours at 37°C resulted in < 10% loss in φX174 RF II DNA as determined by agarose gel electrophoresis.

RNase Assay: Incubation of 10 units of enzyme with 40 ng of 300 bases Fam-labeled RNA at 37°C for 3 hours in a 50 µl reaction buffer results in < 10% of RNA degradation as analyzed from polyacrylamide gel.

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

A Typical Exonuclease V Reaction:
1 µl sample DNA (~ 1 µg)
3 µl NEBuffer 4 (10X)
3 µl 10 mM ATP
y µl H2O (up to final volume of 30 µl)
1 µl Exonuclease V (10 units)
1. Incubate at 37°C for 30 minutes.
2. To stop reaction add EDTA to 11 mM.
3. Heat Inactivation 70°C for 30 minutes.
4. Clean-up treated samples by column purification and/or ethanol precipitation.

Note: Estimate amount of DNA to be removed by agarose gel electrophoresis or OD260. If > 1 µg scale up all reaction components proportionately.
References: