Alkaline Phosphatase (Fast)

REF: EG15208S

Storage Condition

-20℃

Components

Component	Amount
Alkaline Phosphatase (Fast)(1 U/μl)	1000 μl
10× AP Buffer	2×1 ml

Description

Alkaline Phosphatase (Fast) is an enzyme that catalyzes the hydrolysis of the 5' and 3' phosphate groups of DNA, RNA, and nucleotides. It can also remove protein phosphate groups but does not hydrolyze phosphodiester and phosphotriester bonds. This enzyme can dephosphorylate the ends of all types of DNA in 10 minutes at 37℃ . It has 100% activity in CutOne™ buffer and is inactivated by incubation at 80 ℃ for 20 minutes. When used together with LightiNing™ restriction endonucleases, it simplifies the "digestion-dephosphorylation" reaction in one tube.

Definition of Activity Unit

Under the condition of 37℃ in AP buffer, the amount of enzyme required to dephosphorylate the 5' end of 1 μg linearized pUC57 DNA in 10 minutes is defined as one unit.

Quality Control Assays

Endonuclease Activity

A 20 μl reaction in AP Buffer containing 200 ng of supercoiled plasmid and 1 U of Alkaline Phosphatase (Fast) incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction in AP Buffer containing 15 ng of double-stranded DNA fragments and 1 U of Alkaline Phosphatase (Fast) incubated for 16 h at 37℃, and no degradation was detected by agarose gel electrophoresis.

Protocol

1. Synchronized linearization and dephosphorylation of plasmid DNA

① Prepare the following reaction mixture on ice:

Reagent	Amount
Plasmid DNA[a]	1 μg
10× CutOne™ Buffer	2 μl
LightNing™ Restriction Enzyme	1 μl
Alkaline Phosphatase (Fast)	1 U (1 μl)
ddH_2O	To 20 μl

a. To ensure efficient dephosphorylation, the plasmid DNA should be free from RNA and genomic DNA contamination.

② Mix gently and spin down, then incubate at 37℃ for 15~30 min.

Note: Prolonged incubation may result in star activity.

③ Incubate at 80℃ for 20 min to terminate the reaction.

2. Dephosphorylation of linear DNA

This protocol is suitable for removing the phosphate groups from the 3' and 5' ends of DNA.

① Prepare the following reaction mixture on ice:

Reagent	Amount
Linear DNA	1 μg
10× AP Buffer	2 μl
Alkaline Phosphatase (Fast)	1 U (1 μl)
ddH_2O	To 20 μl

② Mix gently and spin down, then incubate at 37℃ for 10 min;

③ Incubate at 80℃ for 20 min to terminate the reaction.

3. Dephosphorylation of protein phosphate groups

① Prepare the following reaction mixture on ice:

Reagent	Amount
10× AP Buffer	2 μl
Phosphorylated protein	2~4 μg (final concentration 0.1~0.2 mg/ml)
Alkaline Phosphatase (Fast)	10 U (10 μl)
ddH_2O	To 20 μl

② Mix gently and spin down, then incubate at 37℃ for 1 hour;

③ Add EDTA to a final concentration of 50 mM or add sodium vanadate (Na_3VO_4) to a final concentration of 10 mM to terminate the reaction.

Note: The above examples are for the reaction mixture and protocol of dephosphorylating protein phosphate groups. Adjust the amount of Alkaline Phosphatase and optimal incubation time based on the specific substrate type when conducting experiments.

Notice

DNA bound with Alkaline Phosphatase may show band shifting or smearing on agarose gel. To avoid this, you can add 6× Loading Buffer containing SDS to the sample, incubate at 80℃ for 20 min, and then cool on ice before electrophoresis.

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