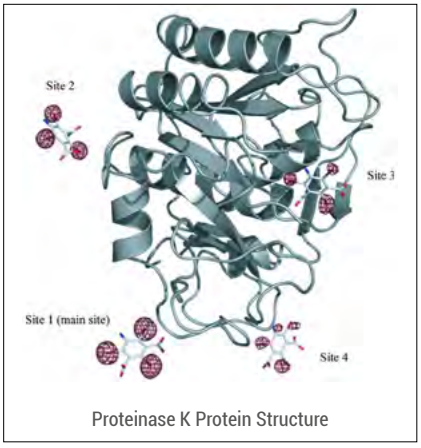
FT-85870n

Proteinase K

Product data



Proteinase K Protein Structure

Proteinase K, from *Tritirachium album timber (Engyodontium album)*
Syn.: peptidase K, Tritirachium alkaline proteinase
Protein K powder #858706
Proteinase K solution #718961

CAS: [39450-01-6] MW: 8,900 daltons (28.9 kDa).
primary sequence for proteinase K:
GAAQTNAPWGLARISSTSPGTSTYYYDESAGQGSCVYVIDTGIEASHPEF
EGRAQMVKTYYYSSRDGNGHGTHCAGTVGSRTYGVAKKTQLFGVKVLDDN
GSGQYSTIIAGMDFVASDKNNRNCPKGVVASLSLGGGYSSSVNSAAARLQ
SSGVMVAVAAGNNNADARNYSPASEPSVCTVGASDRYDRRSSFSNYGSVL
DIFGPGTSILSTWIGGSTRSISGTSMATPHVAGLAAYLMTLGKTTAASAC
RYIADTANKGDLSNIPFGTVNLLAYNNYQA

FAQ & Technical tips

What is Proteinase K?

PProteinase K (also protease K or endopeptidase K) is a broad-spectrum serine protease widely used in molecular biology. Proteinase K is able to digest native keratin (hair), hence, the name "Proteinase K". It is commonly used because of its broad specificity, that makes it useful to clean nucleic acid complexe samples and to lyse cells. It has been used for isolation of mRNA, high molecular weight DNA and to inactivate other enzymatic activities.
The enzyme was discovered in 1974 in extracts of the fungus *Engyodontium album (formerly Tritirachium album)*.

What are proteinase K applications?

Proteinase K is ideal for many molecular biology applications because it is able to break down proteins and inactivate DNases and RNases that would otherwise degrade a desired sample of DNA or RNA.
- Digestion of unwanted proteins in molecular biology applications
- Removal of endotoxins bound to cationic proteins such as lysozyme and RNaseA
- Removal of nucleases for in situ hybridization
- Prion research with respect to TSE (transmissible spongiform encephalopathies)
- Protease footprinting
- Mitochontrial isolation
- Isolation of genomic DNA
- Isolation of cytoplasmic RNA
- Isolation of highly native DNA or RNA

Proteinase K is commonly used in molecular biology to digest protein and remove contamination from preparations of nucleic acid. Addition of Proteinase K to nucleic acid preparations rapidly inactivates nucleases that might otherwise degrade the DNA or RNA during purifis ideal for these applications because proteinase K is able to break down proteins and inactivate DNases and RNases that would otherwise degrade a desired sample of DNA or RNA.Proteinase K is cation. It is highly-suited to this application since the enzyme is active in the presence of chemicals that denature proteins, such as SDS and urea, chelating agents such as EDTA, sulfhydryl reagents, as well as trypsin or chymotrypsin inhibitors. Proteinase K is used for the destruction of proteins in cell lysates (tissue, cell culture cells) and for the release of nucleic acids, since it very effectively inactivates DNases and RNases. Some examples for applications: Proteinase K is very useful in the isolation of highly native, undamaged DNAs or RNAs, since most microbial or mammalian DNases and RNases are rapidly inactivated by the enzyme, particularly in the presence of 0.5 – 1% SDS. Purification of genomic DNA from bacteria (miniprep): bacteria from a saturated liquid culture are lysed and proteins are removed by a digest with 100 µg/ml Proteinase K for 1 h at 37 °C. The enzyme's activity towards native proteins is stimulated by denaturants such as SDS. In contrast, when measured using peptide substrates, denaturants inhibit the enzyme. The reason for this result is that the denaturing agents unfold the protein substrates and make them more accessible to the protease.

See below guidelines for use in main applications.

What is the Enzyme activity of proteinase K?

Proteinase K is a broad-spectrum serine protease under the subtilisin-like class (there are two types of serine proteases, chymotrypsin-like and subtilisin-like). This enzyme belongs to Peptidase family S8.
The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups. This makes ti a broad spectrum proteinase.
Proteinase K is active with or without the presence of SDS and EDTA (to thes points, see other techn.tips).
Activated by calcium (1 – 5 mM), the enzyme digests proteins preferentially after hydrophobic amino acids (aliphatic, aromatic and other hydrophobic amino acids). Although calcium ions do not affect the enzyme activity, they do contribute to its stability. Proteins will be completely digested, if the incubation time is long and the protease concentration high enough. Upon removal of the calcium ions, the stability of the enzyme is reduced, but the proteolytic activity remains. Proteinase K has two binding sites for Ca2+, which is located close to the active center, but is not directly involved in the catalytic mechanism. Removal of the Ca2+ ions reduces the catalytic activity of Proteinase K by 80 %. The residual activity is sufficient to digest proteins, which usually contaminate nucleic acid preparations. Therefore, the digest with Proteinase K for the purification of nucleic acids is performed in the presence of EDTA (inhibition of magnesium-dependent enzymes). If the presence of Ca2+ required, Ca2+ is added up to a concentration of 1 mM and is removed by the addition of EGTA (pH 8.0; final conc. 2 mM) later on.

Buffer (pH 8.0, 50°C, 1.25µg/mL protease K, 15 min incubation)	Proteinase K activity (%)
30 mM Tris·Cl	100
30 mM Tris·Cl; 30 mM EDTA; 5% Tween 20; 0.5% Triton X-100; 800 mM GuHCl	313
36 mM Tris·Cl; 36 mM EDTA; 5% Tween 20; 0.36% Triton X-100; 735 mM GuHCl	301
10 mM Tris·Cl; 25 mM EDTA; 100 mM NaCl; 0.5% SDS	128
10 mM Tris·Cl; 100 mM EDTA; 20 mM NaCl; 1% Sarkosyl	74
10 mM Tris·Cl; 50 mM KCl; 1.5 mM MgCl2; 0.45% Tween 20; 0.5% Triton X-100	106
10 mM Tris·Cl; 100 mM EDTA; 0.5% SDS	120
30 mM Tris·Cl; 10 mM EDTA; 1% SDS	203

Why is proteinase K digestion performed at 50°C? with SDS/urea? What is the best temperature?

The proteinase K activity increases with temperature, up to a certain point. The optimal temperature for activity ranges between 50-65 °C.

Proteinase K activity is greatly increased by addition of denaturing agents like SDS or urea (Hilz et al., 2008), indicating that the denaturation of the substrates helps Proteinase K to degrade them. Increasing the temperature to 50°C will also unfold some proteins already, making it easier for the Proteinase K to degrade them. The proteinase K seems to be a pretty stable enzyme, and can still work at this temperature.
Additionally, proteinase K becomes generally more stable and more active when in buffers that contain these activators.
Proteinase K is active in a pH between 7.5 and 12.0.
Now, optimizing proteinase K activity (by temperature) might not be the most important thing during your procedure. Sometimes, special techniques will require to adapt **the best overall results**. Keep in mind is that
- while the listed range is great for proteinase K activity, the enzyme is still active in temperatures ranging between ~20-65 °C, and having that wide temperature flexibility available might be useful for very particular methods you're performing.
- beyond 55 °C, you risk inactivating proteinase K and surely as temperatures increase >65°C.

What is the quickest most effective way to inactivate proteinase K?

As with most protein enzymes change the temperature (at >55 °C) or change the pH significantly.

Heat is a widely used way of inactivating proteinase K. While the activity of proteinase K increases with temperature, and is optimized at about 55-65 °C, heating proteinase K to 70 - 95 °C. will inactivate it. Keep in mind, however, that heating proteinase K does not fully inactivate the enzyme. There will always be a small amount of activity remaining through this method. Whatever, it is a simple procedure, and we recommend thus 95 °C for 10 minutes
Protease inhibitors such as PMSF and AEBSF (Pefabloc®) can also be used to permanently inactivate proteinase K.

Does EDTA inactivate proteinase K?

Chelators such as EDTA or EGTA don't have a direct effect on proteinase K enzyme activity.
Often, the reason for using EDTA with proteinase K during DNA or RNA purification is for the removal of calcium. But because calcium is related to proteinase K stability, the addition of EDTA can impact the calcium and therefore reduce proteinase K activity to some extent.

Do you have guidelines for using Proteinase K?

1.Isolation of high molecular weight DNA

Chromosomal DNA that has been embedded in agarose plugs can be treated with Proteinase K to inactivate rare-cutting restriction enzymes used to digest the DNA. Proteinase K is used for this method at a concentration of 1mg/mL in a buffer containing 0.5M EDTA and 1% N-lauroylsarcosine (v/v). Incubate 24-48 hours at 37°C.

2.Isolation of plasmid and genomic DNA

Genomic or plasmid DNA can be isolated from liquid nitrogen frozen cells or cultured cells using Proteinase K. Incubate 50-100 mg of tissue or 1×108 cells in 1mL of buffer containing 0.5% SDS (w/v) with Proteinase K at a concentration of 1mg/mL, for 12-18 hours at 50°C.

3.Isolation of RNA

For cytoplasmic RNA isolation, centrifuge the cell lysate, remove the supernate and add 200ug/mL Proteinase K and SDS to 2%(w/v). Incubate for 30 minutes at 37°C. Total RNA can be isolated by passing the lysate through a needle fitted to a syringe prior Proteinase K treatment.

4.Inactivation of RNases , DNases and enzymes in reactions

Proteinase K is active in a wide variety of buffers (see FAQ "What is the Proteinase K activity in commonly used buffers?"). The enzyme should be used at a ratio of approximately 1:50 (w/w, proteinase K:enzyme). Incubation is at 37°C for 30 minutes.

How to determine if the proteinase K is working?

One can use an artificial substrate like benzoyl arginine -p-nitroanilide that when cleaved by the proteinase yields a yellow colored p-nitroaniline that absorbs at ~ 410 nm. You can then determine the activity of the proteinase K by determining how many micromoles of the p-nitroanilide are produced per minute. The by dividing by the total amount of protein in the solution you can determine the specific activity of the enzyme activity = units (one unit equals 1 mole of p-nitroanilide produced /min), specific activity = units of enzyme activity /mg total protein. Alternatively, prepare a 1.25 % agar containing 2% casein in pH eight buffer and pour into a petri dish. Punch 4mm diameter wells in the gel about 20 mm apart. In the wells place various concentrations of your proteinase K solution. Allow to incubate at room temp (humidified)for 6-8 hrs. Look for the clear zones around the wells. The size of the clear zone is proportional to the concentration of the proteinase K and gives a visual appraisal of active digestion of a protein rather than a synthetic substrate.

What exactly is the relationship between proteinase K and calcium?

Proteinase K binds to two Ca 2+ ions which help maintain the stability of the enzyme, especially when it's subjected to increasing temperatures. Calcium also protects proteinase K from autolysis. While calcium helps maintain proteinase K thermostability, it is not necessary for proteolytic activity.

According to Richard Tullis and Harvey Rubin, this relationship becomes more interesting when DNase I is involved. Proteinase K is known to inactivate DNases and RNases, but when DNase I is in the presence of Ca2+, it is protected from proteinase K (concentration of 1mg/mL). RNase, on the other hand, is inactivated whether or not it is in the presence of Ca2+. Their findings suggest a method for treating contaminated RNase free DNaseI or isolating highly polymerized RNA.

What is the function of proteinase K in DNA extraction?

During the extraction of DNA (or nucleic acids in general), there is a lot of contaminating proteins present. These contaminants must be removed. Proteinase K, which is a broad spectrum serine protease, is used in many DNA extraction protocols to digest these contaminating proteins.

In addition, there may be nucleases (enzymes that degrade nucleic acids) present. The addition of proteinase K degrades these nucleases and protects the nucleic acids from nuclease attack. In addition, proteinase K is stable over a wide pH range and is well suited for use in DNA extraction.

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How is proteinase K involved with cell lysis?

To answer this question, the first thing to understand is that proteinase K is a broad-spectrum protease capable of digesting a wide range of native proteins.

When it comes to cell lysis, particularly for downstream DNA isolation and purification, proteinase K can be part of the lysis step by digesting surface proteins. Further into the procedure, when it comes time to resuspend and lyse the nuclei in a buffer containing proteinase K, the proteinase K will help digest proteins that would otherwise degrade the sample.

Why do many DNA extraction lysis buffer recipes call for proteinase K and RNase?

First of all, you want RNase to be added because it would break down contaminating RNA during your DNA isolation. And you want to use proteinase K because it will break down damaging proteins, DNases and … RNases!

You thus will understand that combining proteinase K and RNase is needed but a question of timing and optimization. Some researchers suggest adding the RNase in first, allowing time for it to work. Then proteinase K and SDS can be added to break down unwanted proteins. Something else to consider is that some protocols will have you incubate in SDS, proteinase K and RNase at 37 ˚C for a certain amount of time. Since proteinase K activity is not as highly optimized at this temperature, this likely gives RNase time to work. Later steps in the protocol suggest a second incubation at 55 ˚C for a longer period of time, which would be a more optimal temperature for proteinase K activity, allowing it to digest other unwanted proteins.

How should Proteinase K be stored/ What is the shelf-life of Proteinase K?

Please refer to storage conditions of your provided, e.g. Protein K powder #858706 ; Proteinase K solution #718961

The proteinase K is a pretty stable enzyme, so lyophilized form and even solution can be stored at RT for short-medium time. We however recommend storing Proteinase K at 4 degrees Celsius for long-term storage, but leaving it out for a few days at room temperature should not ruin the reagent.

Typically once vial is opened,
- Stock Solution: aliquot your stock solution and store at -20 °C for up to 1 year.
- Lyophilized Powder: Store desiccated at -20 °C for up to 2 years.
When you should include proteinase K in a kit, consider that storage and shipping conditions may be more demanding than expected (duration >6months, temperature >30°C…), and in particular if you repack or formulate proteinase K. In such case, you should have to really test the proteinase K stability in your application / in you kit.
May you provide us the stability result, I will be happy to thank you with a discount on your next order: this could be helpful for other customers!

How do you make proteinase K stock solution?
Proteinase K is very water soluble (1mg/mL), yielding a clear colorless solution.
Dissolve protein K as 20mg/mL stock solution using Tris buffer and $CaCl_2$, as follows:
100mg 5mL (20mg/mL)
500mg 25mL (20mg/mL
1g 50mL (20mg/mL)
Other possible buffers are Tris or PBS. When working with PBS, however, it can be a little tricky, which is possibly due to the pH (still within optimal range, but on the lower end of that range). Typically, adding proteinase K powder a little at a time while mixing into solution will help dissolve it into PBS.
You may gain time and reproducibility using Proteinase K solution #718961.

Is there an alternative to using proteinase K?
- Extractions
if this question is specifically geared toward isolating DNA from other proteins, the phenol-chloroform extraction is another option useful for removing proteins from solution. However, this method is more toxic. The benefit of using proteinase K during DNA extraction is its ability to degrade a wide range of damaging.
- digesting surface proteins on the cell membrane.
Trypsin is more popular than proteinase K for this application (to detach cells from culture wares). It however suffers from several drowbacks (too harsh / reduce cell viability; bovine contaminent; …).
A smarter alternative is thus Accutase #N68081.

What does proteinase K have to do with prion diseases or TSE?
Proteinase K is involved in differentiating between the normal PrP C (prion protein / protease-resistant protein) and PrPSC (disease causing isoform). Both PrPC and PrPSC have the same molecular weight, however, PrPSC is resistant to proteinase K. Samples, which might contain both, are treated with proteinase K, which will eliminate PrPC and convert PrPSC into PrPRES which has a lower molecular weight and can be pelleted, and therefore distinguished.

References

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- Wikipedia.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

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