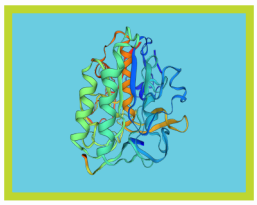
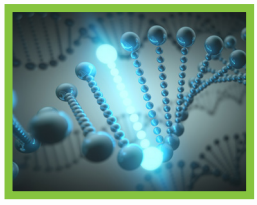
GOLDBIO®

PROTEINASE K HANDBOOK


DISCOVER MORE

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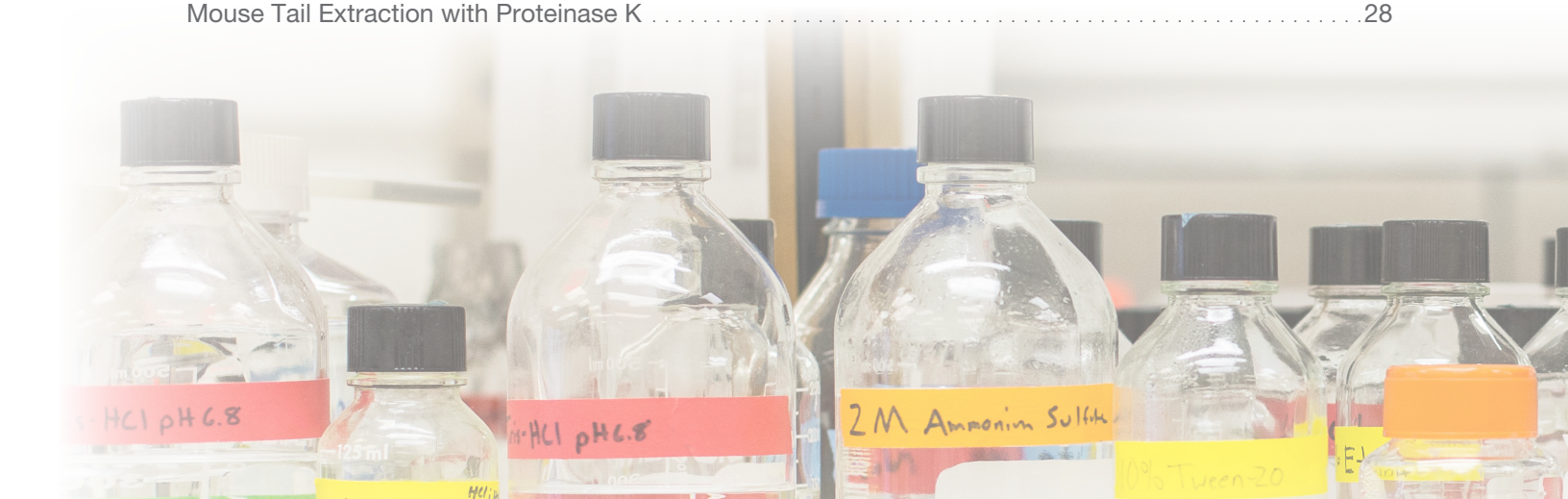


Proteinase K Handbook
By | Karen Martin, Tyas Kroemer, Chris Menne, Adriana Richardson, Chris Harper, and Fernanda Ruiz
Edited | Shaun Kennedy, Karen Pierce, Adriana Richardson, Tyas Kroemer
2020 | Gold Biotechnology ®

Introduction

Discover articles and illustrations familiarizing yourself with the enzyme proteinase K.

In this section, you'll learn about what proteases are, what proteinase K is, related applications and detailed product information.

What is a Protease

by Tyas Kroemer, PhD

What is a protease?

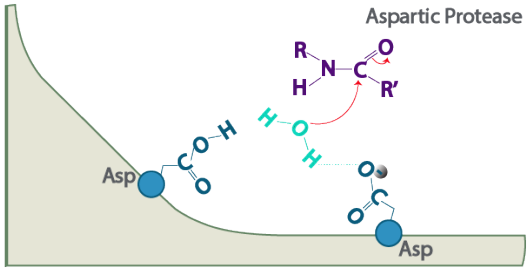
Proteases are important reagents in biotechnology and molecular biology laboratories. The use of proteases is often necessary to inactivate protein contaminants in our prepared samples.

Proteases are enzymes that catalyze the cleavage of a protein into amino acids. These enzymes digest a protein by using an amino residue or an activated water molecule as a nucleophile. A nucleophile is an atom of an amino acid that donates an electron to another molecule, causing changes in another amino acid.

Protease Mechanisms

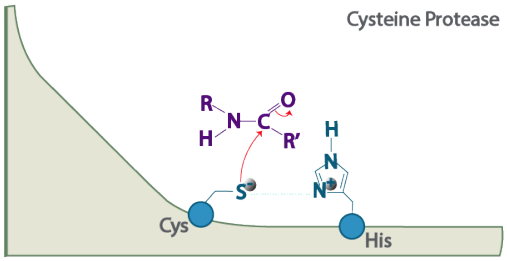
Proteases use two types of mechanisms to break a peptide bond:

1. The first mechanism for peptide bond breaking involves the use of an activated water molecule to perform a nucleophilic attack. Examples for these enzymes are aspartic-, glutamic, and metallo proteases.



2. The second mechanism involves the use of a nucleophilic residue to cleave a peptide bond. Serine proteases use an active serine to perform a nucleophilic attack.

Cysteine peptidases use the sulfur in cysteine to perform nucleophilic attack. Threonine proteases use a threonine residue as a nucleophile.

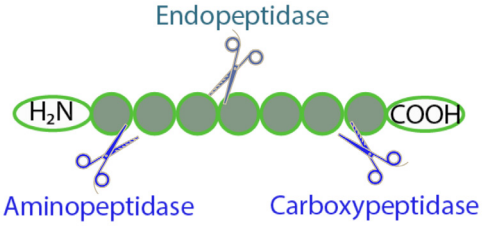


The classes of proteases

Proteases can be divided into different classes based on the location of their cut, chemical structures, or catalytic mechanism.

Based on the cleavage site, proteases can be classified into:

- Endopeptidases: these enzymes cut internal peptide bonds.
- Exopeptidases: their catalytic activity is near the amino- or carboxy-termini of their substrates.



Different Classes of Peptidases. *The endopeptidase breaks internal peptide bonds. The aminopeptidase cuts at the amino terminus of a substrate, whereas carboxypeptidase cleaves near a carboxy terminus of a substrate.*

However, there is another way to classify proteases. Based on their chemical structures and catalytic mechanisms, there are six classes of proteases:

- **Aspartic proteases:** using an aspartatic acid in their catalytic mechanism.
- **Glutamic proteases:** using a glutamatic acid in their mechanism.
- **Metalloproteases:** using a metal ion to catalyze the reaction.
- **Cysteine proteases:** using a cysteine as a nucleophile.
- **Threonine proteases:** using a threonine in the catalytic mechanism.
- **Asparagine peptide lyases:** using an asparagine for an elimination reaction.
- **Serine proteases:** using a serine as a nucleophile.

Serine proteases

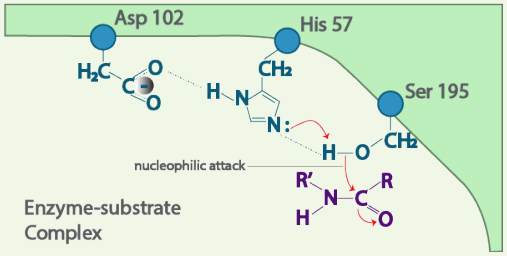
Serine proteases are proteases characterized by the presence of a reactive serine residue in **a catalytic triad**. A catalytic triad is a group of three amino acids at the enzyme active site that are involved in catalysis. The catalytic triad allows the transfer of protons into and out of the active site. An example of a catalytic triad for chymotrypsin: **Ser 195**, **His 57**, and **Asp 102**. In this example, the three-letter words represent the amino acid, and the number represents their position.

In these serine proteases, the catalytic triad usually consists of serine, aspartic acid, and histidine amino acids. Some other groups of serine proteases may have a different combination of residues in the catalytic triad, such as Ser-His-Glu, Ser-Lys/His, or His-Ser-His. However, the homology studies and analyses of the active site among serine proteases show that all of these enzymes have a nucleophile serine and the same catalytic mechanism.

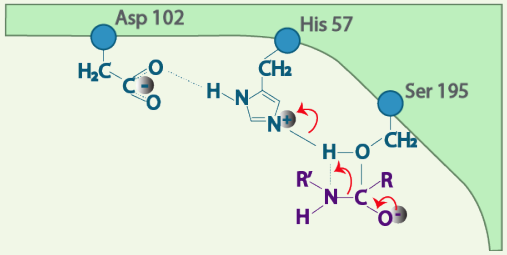
The catalytic mechanism of the serine protease chymotrypsin usually represents the catalytic mechanism for all of serine proteases. It is because the structure and mechanism of chymotrypsin is well characterized. Chymotrypsin catalyzes the cleavage of peptide bonds of proteins near the carboxyl group of the aromatic amino acid. The catalytic triad of chymotrypsin consists of **Ser 195**, **His 57**, and **Asp 102.**

The step-wise catalytic mechanism of chymotrypsin

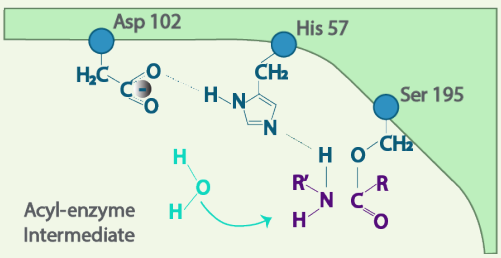
1. The active Serine (Ser 195) performs the nucleophilic attack on the carbonyl carbon atom of the peptide.



2. The nucleophilic attack causes the formation of tetrahedral intermediate.

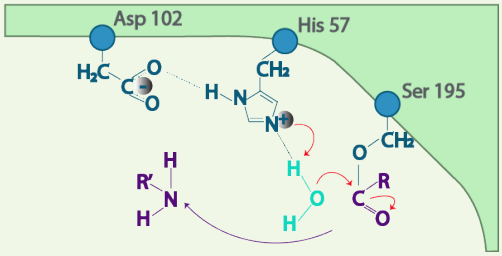


3. The tetrahedral intermediate changes into an acyl-enzyme intermediate. The solvent water comes to replace the first amine product.

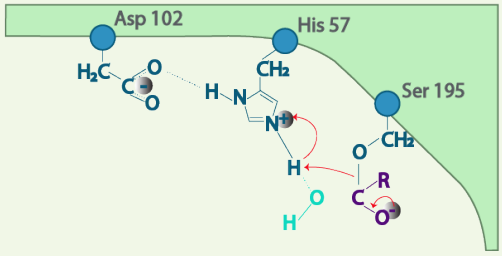


The step-wise catalytic mechanism of chymotrypsin (continued)

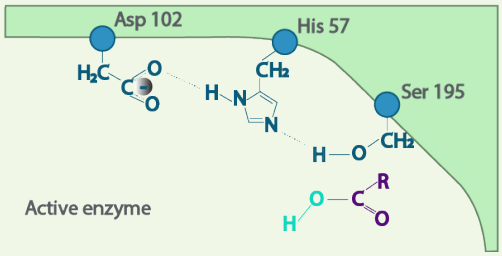
4. The first amine product leaves and the solvent water replaces it.



5. The reaction produces a second tetrahedral intermediate.



6. The final reaction causes the release of the second amine product and the formation of the active enzyme.



The classes of serine proteases

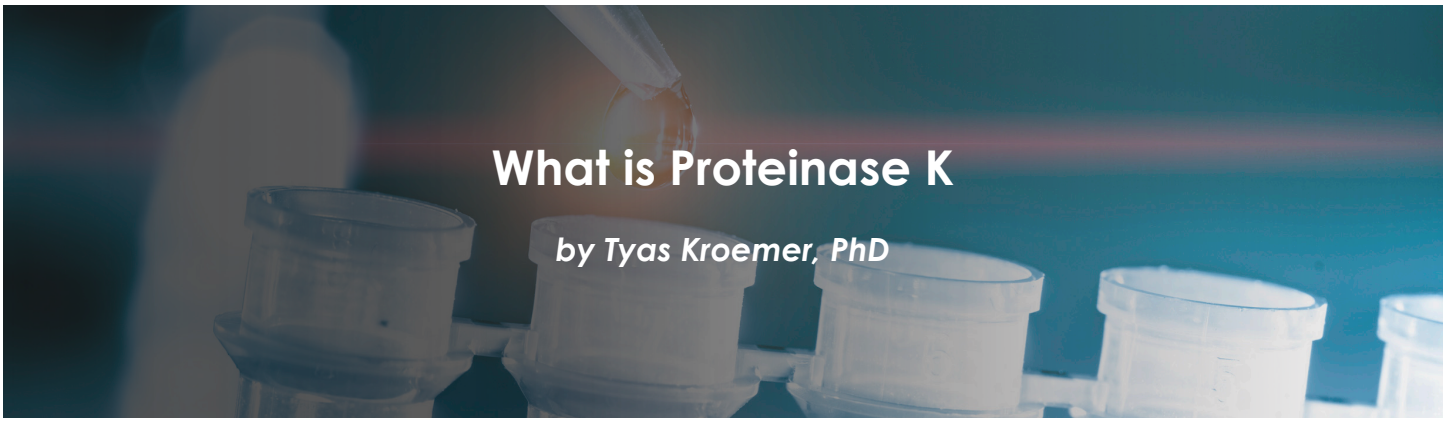
Serine protease consists of large class of proteases based on their structural similarity and functions. Below are some well-characterized groups of serine proteases:

Chymotrypsin: Chymotrypsin has a specificity pocket with hydrophobic amino acid, therefore it attracts substrate proteins with hydrophobic amino acids. A specificity pocket is a pocket at the enzyme active site allowing a substrate to bind for cleavage.

Trypsin: Trypsin has a negatively charged aspartic acid in the pocket, attracting a positively charged amino acid.

Elastase: Members of elastase group have a small specificity pocket, so only a small amino acid enters it.

Subtilisin: Members of this group show high specificity for aromatic and hydrophobic amino acids. Although chymotrypsin and subtilisin have different primary and tertiary structures, the catalytic triad of subtilisin resembles that of chymotrypsin.



What is Proteinase K

by Tyas Kroemer, PhD

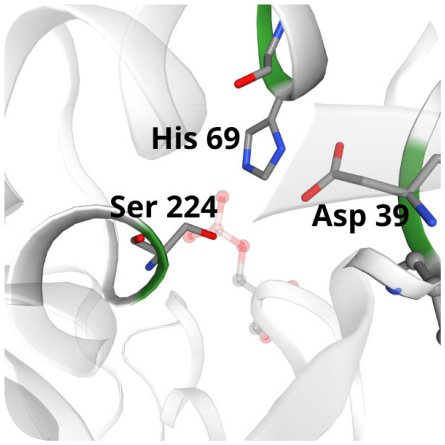
What is proteinase K?

Proteinase K is an endopeptidase belonging to the subtilisin group of serine proteases. In molecular biology laboratories, proteinase K is useful during preparation of DNA or RNA samples by degrading and inactivating proteins.

Proteinase K has five cysteines. Four of the cysteine residues form two disulfide bonds (34-124 and 179-248, respectively) and one lies below one of the catalytic triads. Proteinase K often binds two calcium ions required for full enzymatic activity.

The chemical structure of proteinase K

Proteinase K has the catalytic triad consisting of **Ser 224**, **His 69**, and **Asp 39**. The substrate recognition sites are two peptide chains, 99-104 and 132-136. It also has a free Cys 73 near His 69.



Catalytic Triad of Proteinase K from P. album. *Catalytic triad model is created with SWISS-MODEL (https://swissmodel.expasy.org).*

General questions about proteinase K

Why is proteinase K used for mammalian DNA isolation?

After the addition of a detergent, all sorts of material is released into lysate including harmful nucleases. To protect DNA from degradation, a protease, in this case proteinase K is used. The reason proteinase K is a good choice is because it's pretty stable at higher temperatures and it's broad-spectrum in terms of its targets.

How is proteinase K used for mammalian DNA isolation?

The first step for DNA isolation from mammalian cells and leucocytes is lysing the cells and spinning them down to collect a pellet. Once in suspension, detergent (SDS) is added to disrupt the nuclear membrane. Both histone and non-histone proteins release the DNA after digestion of protein by proteinase K.In addition, proteinase K digests and inactivates ribonuclease in the solution.

After digestion by proteinase K, phenol extractions separate DNA from the lipids and proteins. Hydrophobic lipids stay in the organic phase, whereas the DNA resides in the aqueous phase. Protein (including proteinase K) stays at the organic/aqueous interphase. DNA is extracted from the aqueous layer.Phenol extractions are important to eliminate proteinase K from the high molecular weight DNA.

Can proteinase K be used for RNA isolation?

During RNA isolation, the addition of proteinase K degrades and inactivates even tiny amounts of ribonuclease in the presence of substrate. The additional phenol chloroform and isopropanol precipitation step reduces protein contamination and makes the final pellet easier to re-dissolve.

Proteinase K Product Description

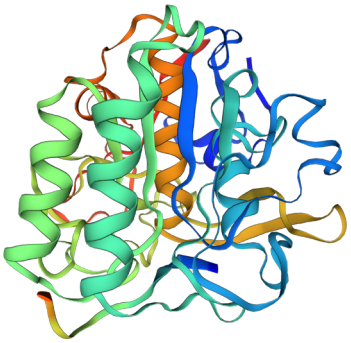


PHOTO | model is created with SWISS-MODEL (https://swissmodel.expasy.org)

Proteinase K is a highly reactive nonspecific serine protease that belongs to the subtilisin family of proteins. It cleaves at the carboxylic acid side of aliphatic, aromatic, or hydrophobic amino acids. Proteinase K is capable of inactivating RNases and DNases and is used in the isolation or preparation of high molecular weight nucleic acids. Proteinase K is also useful for helping to characterize enzymes, due to its cleavage specificity. This enzyme was designated proteinase K because of its ability to hydrolyze keratin. Proteinase K is stable in a wide variety of detergents and buffer salts and at various temperatures and pH. The isoelectric point of proteinase K is 8.9.

- Typically used at a concentration of 50-100 µg/ml.
- Active with or without the presence of SDS, urea, EDTA or various metal ions, but the activity of proteinase K can be increased by adding the denaturing agents and the structure of proteinase K can be stabilized by addition of Ca 2+.
- Inactivated by heating to 95°C for 10 minutes or using an inhibitor such as PMSF, AEBSF or DFP.

Proteinase K, RNase/DNase free

P-480-100	100 mg
P-480-500	500 mg
P-480-1	1 g
P-480-2	2 x 1 g
P-480-3	3 x 1 g
P-480-4	4 x 1 g
P-480-5	5 x 1 g

Proteinase K Solution 20 mg/ml, RNase/DNase free

P-480-SL2	2 mL
P-480-SL4	4 mL (2 x 2 mL)
P-480-SL10	10 mL (5 x 2 mL)

Specifications	**Grade:**	Molecular Biology Grade
	Weight:	28.5 kDa
	Activity:	≥30 U/mg
Source	Yeast cells with cloned gene encoding Engyodontium album (Tritirachium album) endolytic protease.	
Storage/Handling	Store at -20°C. Powder: Reconstitute in 50mM Tris-HCl (pH 8.0), 3mM CaCl2. Solution: Proteinase K Solution is provided in 50mM Tris-HCl (pH 8.0), 3mM CaCl2 and 50% glycerol.	

Proteinase K Analysis and Results

Introduction

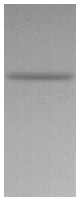
GoldBio Proteinase K is thoroughly tested to ensure that it is highest grade available for your research. Below are a selection of gel images and tables showing the purity and efficacy of GoldBio Proteinase K.

Related Products

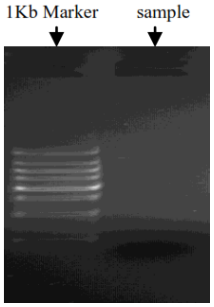
Proteinase K (GoldBio Catalog # P-480)
Proteinase K Solution 20 mg/ml (GoldBio Catalog # P-480-SL)

Analysis

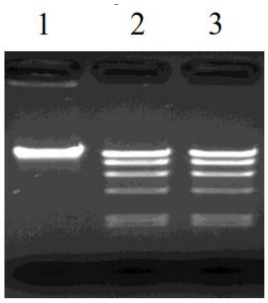
1. SDS-Page



2. DNA and RNA Contaminants assay: none detectable

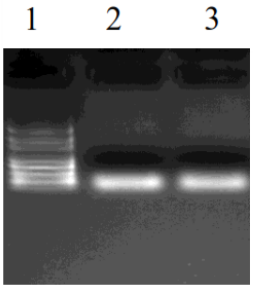


3. DNase Contaminants assay: none detectable



1. λ DNA；2. λ DNA+HindⅢ；3. λ DNA+HindⅢ+Proteinase K

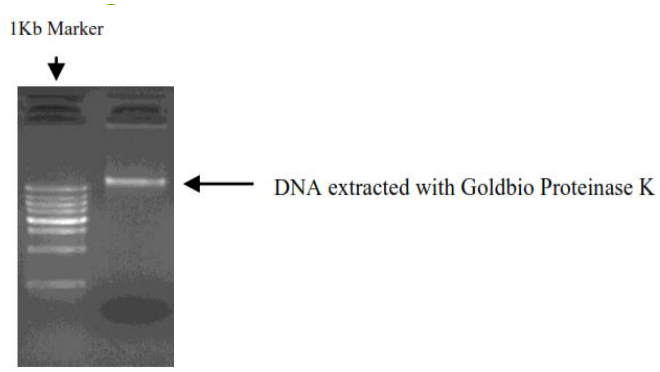
4. RNase Contaminants assay: none detectable



1. DL2000 plus DNA Marker；2. RNA；3. RNA+Proteinase K

(DL2000 plus DNA Marker： 100bp, 250bp, 500bp, 750bp, 1000bp, 2000bp, 3000bp, 5000bp)

5. 20μL avian whole blood genomic DNA extraction



(1Kb Marker:10000、8000、6000、5000、4000、3000、2000、1000bp)

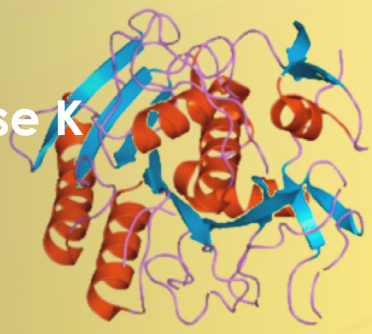
20 μL Avian Whole Blood Genomic DNA Extraction	OD260	OD280	OD260/280	Concentration (μg/ml)	Yield (μg)
GoldBio Proteinase K	0.254	0.130	1.95	101.60	10.16

Application

In this section, you'll learn about enzymatic activity, where RNase contamination occurs and how to prevent it, and you'll get resources to guide you through optimizing your proteinase K digestion.

Judging the Quality of Proteinase K

by Karen Martin



Often, enzymes such as proteinase K are evaluated for their activity. Since the role of an enzyme is to hasten a chemical reaction, understanding enzymatic activity gives researchers a better idea of how well it will perform. The second component for evaluating the quality of proteinase K is looking at its purity. Contaminants such as RNase could dramatically reduce the efficiency of an experiment. In this article, we'll take a closer look at enzymatic activity and purity as ways of judging the quality of proteinase K.

Enzymatic Activity

Rather than its mass, an enzyme is evaluated for its activity and function. A way this is measured is in terms of enzyme units, which describes enzymatic activity. These units are expressed in international units (IU), and it refers to the amount of enzyme needed to convert 1 µmol of substrate to a given product in 1 minute.

An enzymatic reaction occurs in three steps:

1. The enzyme and substrate are mixed together.
2. The substrate binds to the enzyme's active site forming an enzyme-substrate complex.
3. The reaction occurs forming a product dissociated from the enzyme.

Therefore, activity looks at an enzyme's ability to speed up the reaction rate, which is one of the most important characteristics in judging an enzyme.

Higher enzymatic activity in your reagent means you need less of that reagent. The same goes with proteinase K. The more active it is, the less you use.

There are more considerations for activity to keep in mind when using proteinase K.

Proteinase K Purity

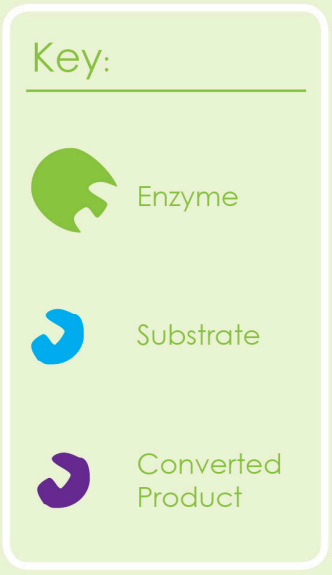
Proteinase K is a protease, which is often used during DNA and RNA isolation. During DNA isolation, proteinase K protects DNA from destructive nucleases. It also degrades proteins in DNA and RNA mixtures, for example a protein capsid, which helps with the extraction process.

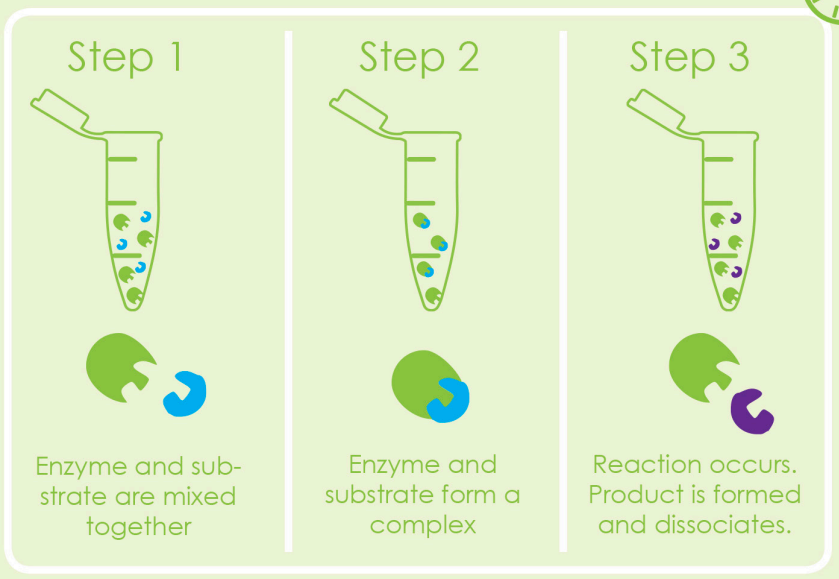
Because of its broad-spectrum nature, proteinase K digests contaminating proteins and nucleases.

Therefore, the purity of proteinase K is important because it impacts its enzymatic activity. Using a proteinase K that is free of DNases and RNases ensures your DNA extraction will yield greater results. Proteinase K specifically tested and cleared of any RNase activity is ideal for your extraction. Be sure to read over your vendor's certificate of analysis to determine the purity of proteinase K.

Enzymatic Activity

Activity looks at an enzyme's ability to speed up the reaction rate. It refers to the **amount of enzyme needed to convert 1µmol of substrate to a given product in 1 minute.**





Preventing RNase Contamination

by Karen Martin

RNase is a nuclease, which is an enzyme that breaks down nucleic acids such as RNA and DNA into smaller units. Nucleases are present in every cell, and upon lysis during an extraction procedure, these enzymes come into contact with precious DNA and RNA.

Preventing RNase contamination ensures your proteinase K works optimally right from the beginning.

Sources of RNase contamination

In order to prevent RNase contamination, let's first look at common places where contamination is likely to happen:

- Your hands or direct skin contact
- Pipette tips and tubes
- Environment and surfaces
- Reagents not specifically for RNA or DNA extraction
- Samples

Preventing RNase contamination

Even if the reagents you bought are RNase-free, the listed channels of contamination could introduce RNase into your procedure. In order to minimize the risk of RNase contamination, here are a few best-practices when working with RNA or DNA.

- Wear gloves always. Don't be afraid to change them often, especially after you have touched your phone, face or any other part of your skin or hair.
- Use RNase-free pipette tips and tubes. Autoclaved tips are not guaranteed to be free of RNase contamination.
- If possible, designate a special set of pipettes for extraction.
- Designate special glass and plastic ware solely for extraction
- Designate a special stock of reagents solely for your extraction procedures. These reagents are not to be used for anything else but extraction.
- Aliquot your extraction reagents and discard them after you have used them.
- Clean working surfaces thoroughly.
- Properly decontaminate all labware such as glassware and plastics. Heat glassware at 300°C for at least four hours. Polystyrene can be decontaminated with a 10 minute 3% hydrogen peroxide soak followed by a deep rinse using RNase-free water.

Important Proteinase K Protocol Tips

by Karen Martin

When is proteinase K used?

Proteinase K is used mostly in DNA and RNA extraction protocols. You'll often find the proteinase K step within the lysis section of the protocol. For example, in the nucleic acid extraction protocol, proteinase K is added to cell lysate and then an incubation period follows to ensure a complete digestion.

To prevent potential digestion of your samples, proteinase K is inactivated after incubation. The common temperature for inactivation is 95°C.

Even in the typical mouse-tail protocol, proteinase K is regularly used to inhibit harmful nucleases. And the addition of proteinase K occurs during the digestion step. The use of EDTA is also suggested to help the inactivation of nucleases by inhibiting Mg^{2+} dependent nucleases.

Determining if digestion happened

Usually, the biggest tell that complete digestion has occurred is that you should see a clear lysed cell solution. If you are not seeing a clear solution after the initial digestion period, extend your incubation time.

Be very careful with this. If you are using a faster method for isolation, especially involving higher volumes of proteinase K, you'll need to pay close attention during proteinase K digestion. A longer digestion may cause degradation of your DNA.

Incubation time

The incubation period with proteinase K is going to depend primarily on the type of sample you're working with. After doing quite a bit of research, here is the range of times we found for different cell and tissue samples. Please keep in mind that your experiment may have different requirements or variables that could greatly influence digestion times, and therefore we strongly encourage you to do your own research before carrying out your work.

Formalin-Fixed Paraffin-Embedded Tissues – Digest for several hours to overnight.

Bacteria – Digest with proteinase K between 1-3 hours. Digestion temperature may also influence how long your digestion should take.

Mammalian cells – There are several papers out there with a wide range of stated digestion times – as little as 1 hour and as long as twelve hours. This is partly due to experimental objectives and the type of cells used. Digestion temperature and proteinase K volumes also have some influence.

Temperatures for digestion

It is strongly encouraged you do more research when optimizing proteinase K. We offer this only as a guide.

FFPE Tissue – Digestion temperatures 55-56°C. Most publications are fairly consistent with this range.

Bacteria – Digestions are often carried out at 55°C. Some publications do state a 37°C digestion temperature. Keep in mind the requirements of the type of sample you're working with and other factors of your experiment.

Mammalian – Publications greatly varied in digestion temperatures. Shorter digestion periods usually correlated with higher temperatures (optimal proteinase K digestion temperatures for mammalian cells range between 50-65°C). Papers with digestions taking place for several hours to overnight usually suggested 37°C. Several other factors can impact the digestion temperature such as cell type (blood, buccal, etc.) and molecular weight.

Proteinase K Protocol Digestion Guide Source: Bacteria

Source	*Setup*	*Comments*	*Quick References*
Bacteria	**Temperature:** 55°C **Time:** 3 hours **Stock Concentration:** 20 mg/ml **Volume:** NA		A Simple "Universal" DNA Extraction Procedure Using SDS and Proteinase K Is Compatible with Direct PCR Amplification
Bacteria	**Temperature:** 55°C **Time:** 1 hour **Stock Concentration:** 20 mg/ml **Volume:** 10 µl		DNA Isolation Protocols Affect the Detection Limit of PCR Approaches of Bacteria in Samples from the Human Gastrointestinal Tract
Bacteria	**Temperature:** 37°C **Time:** 1 hour **Stock Concentration:** 20 mg/ml **Volume:** 3 µl		Preparation of Genomic DNA from Bacteria

Proteinase K Protocol Digestion Guide Source: FFPE Tissue

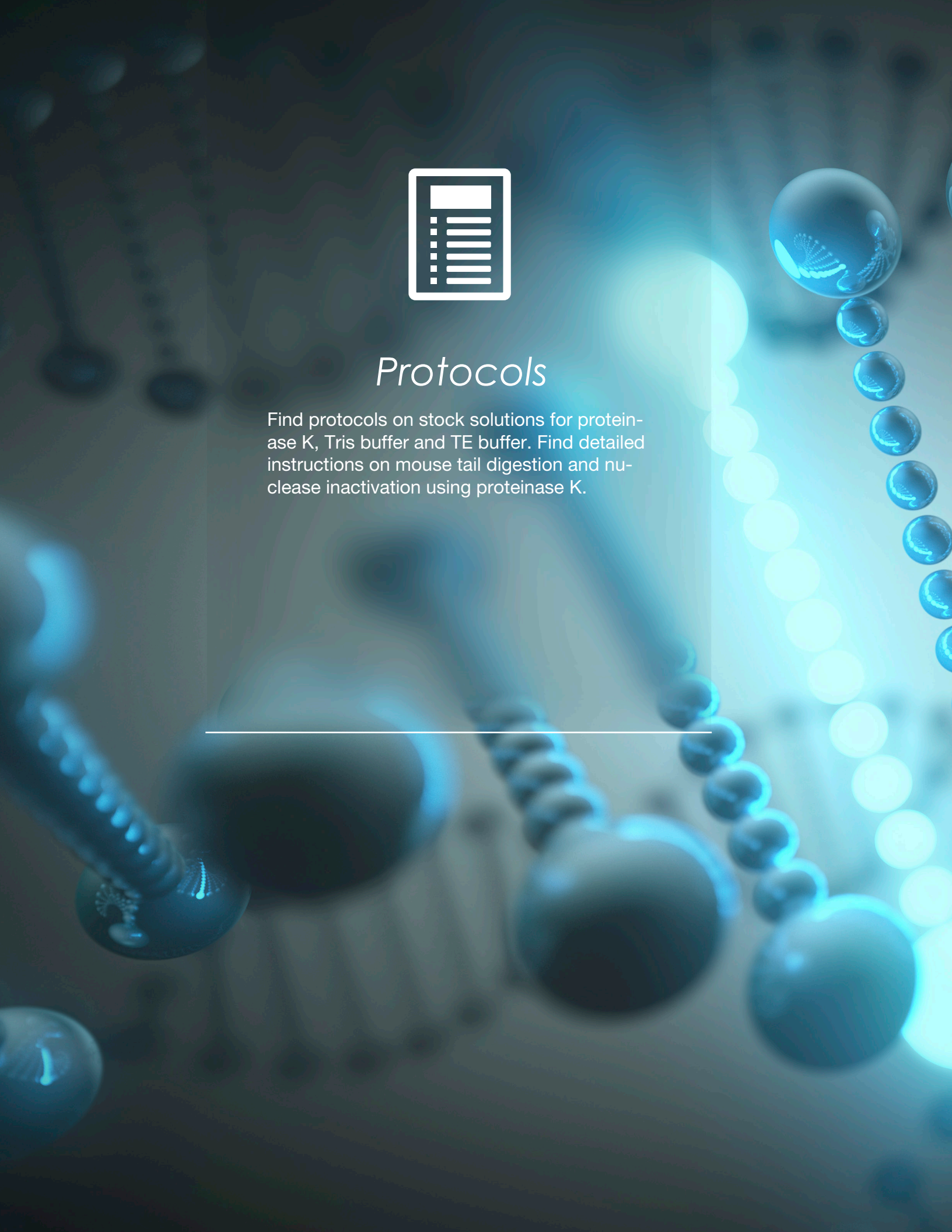
Source	Setup	Comments	Quick References
FFPE Tissue	**Temperature:** 55-56°C **Time:** overnight **Stock Concentration:** 10 mg/ml **Volume:** 40 µl		Comparison of Three Methods for DNA Extraction from Paraf-fin-Embedded Tissues
FFPE Tissue	**Temperature:** 55°C **Time:** 1 hour **Stock Concentration:** 20 mg/ml **Volume:** 10 µl	Method involving kit	Comparison of Three Methods for DNA Extraction from Paraf-fin-Embedded Tissues
FFPE Tissue	**Temperature:** 56°C **Time:** overnight **Stock Concentration:** NA **Volume:** 35 µl		The Suitability of DNA Extracted from Formalin-Fixed, Paraf-fin-Embedded Tissues for Double Differential Polymerase Chain Reaction Analysis
FFPE Tissue	**Temperature:** 56°C **Time:** overnight **Stock Concentration:** NA **Volume:** 35 µl	Method 1 by manufacturer's protocol	Efficient DNA Extraction for HPV Genotyping in Formalin-Fixed, Paraffin-Embedded Tissues
FFPE Tissue	**Temperature:** 65°C **Time:** 16 hours **Stock Concentration:** NA **Volume:** NA	Method 2 by a high heat treatment	Efficient DNA Extraction for HPV Genotyping in Formalin-Fixed, Paraffin-Embedded Tissues
FFPE Tissue	**Temperature:** 55°C **Time:** overnight **Stock Concentration:** 600 mAU/ml **Volume:** 20 µl		Rapid Detection and Identification of Mucormycetes from Culture and Tissue Samples by Use of High-Resolution Melt Analysis

Proteinase K Protocol Digestion Guide Source: Mammalian

Source	*Setup*	*Comments*	*Quick References*
Mammalian	**Temperature:** 37°C **Time:** 12 hours **Stock Concentration:** 50 ug/ml **Volume:** NA	High molecular weight DNA. There are 2 phenol extractions in this paper.	Isolation of High-Molecular-Weight DNA from Mammalian Cells
Mammalian	**Temperature:** 65°C **Time:** 2 hours **Stock Concentration:** NA **Volume:** NA	Human genomic DNA extraction.	High-Yield Noninvasive Human Genomic DNA Isolation Method for Genetic Studies in Geographically Dispersed Families and Populations
Mammalian	T**emperature:** 60°C **Time:** 10-15 minutes **Stock Concentration:** 10 mg/ml **Volume:** 125 µl	Rapid Method Protocol. Perform digestion until clear. Watch carefully. Longer incubation can cause the degradation of DNA.	A rapid method for the isolation of genomic DNA from citrated whole blood
Mammalian	**Temperature:** NA **Time:** overnight **Stock Concentration:** NA **Volume:** NA	Standard isolation for white blood cells.	A rapid method for the isolation of genomic DNA from citrated whole blood
Mammalian	**Temperature:** 58°C **Time:** 2 hours **Stock Concentration:** 20 mg/ml **Volume:** 35 µl	Genomic DNA from buccal cells.	A Simple Mouthwash Method for Obtaining Genomic DNA in Molecular Epidemiological Studies

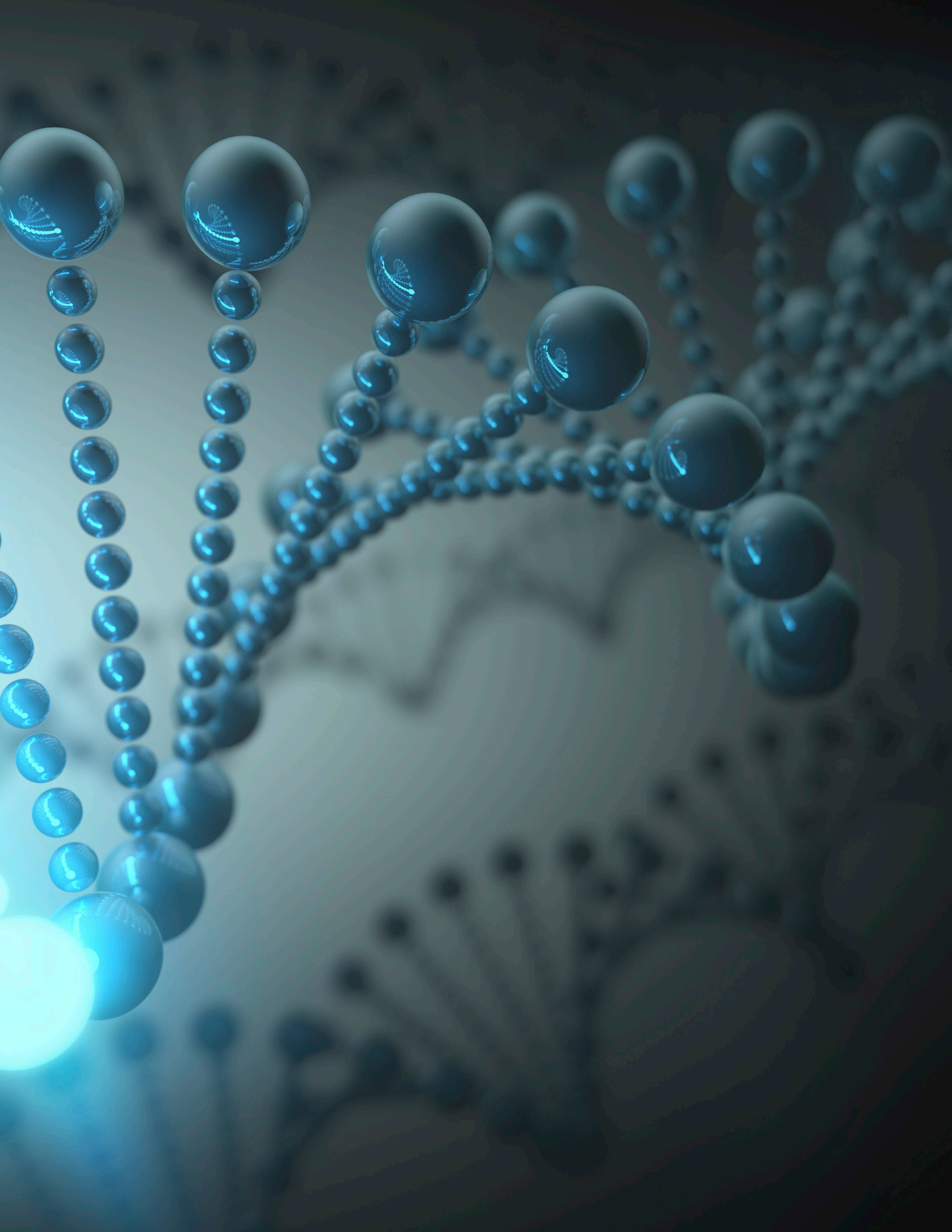
Proteinase K Protocol Digestion Guide Source: Tissue or Blood

Source	Setup	Comments	Quick References
Tissue	**Temperature:** 37°C **Time:** 6 hours-2 days **Stock Concentration:** 10 mg/ml **Volume:** 30 µl	Fresh or Frozen Tissue Protocol.	DNA Extraction from Fresh or Frozen Tissues
Tissue	**Temperature:** 55°C **Time:** 3 hours **Stock Concentration:** 10 mg/ml **Volume:** 30 µl	Fresh or Frozen Tissue Protocol - Alter-native Pro K digestion offered.	DNA Extraction from Fresh or Frozen Tissues
Tissue	**Temperature:** 65°C **Time:** 3 hours **Stock Concentration:** 10 mg/ml **Volume:** 20 µl		Protocol for Extraction of Genomic DNA from Swine Solid Tissues
Tissue or Blood	**Temperature:** 60°C **Time:** 4 hours **Stock Concentration:** 20 mg/ml **Volume:** 20 µl	Second addition: 10 ul of Pro K for muscle, frog toes, lizard tails. Liver requires 20 ul Pro K in the first addition and 5 ul in second. Blood requires 20 ul Pro K in the first digestion and 20 ul Pro K in the second. The additions can be reversed for convenience according to this paper. Second digestion: 37 °C overnight, 20 mg/ml, 5-10 ul.	DNA Isolation from Blood or Tissue Using Phenol/Chloroform



Protocols

Find protocols on stock solutions for proteinase K, Tris buffer and TE buffer. Find detailed instructions on mouse tail digestion and nuclease inactivation using proteinase K.



Proteinase K Stock Solution - 20 mg/ml

Instructions

1. Measure 5 ml of 200mM Tris Buffer (pH 8.0), RNase free* (See the Tris Buffer Stock Solution protocol).
2. Add 1.66 mg (3mM) calcium chloride ($CaCl_2$) to the Tris Buffer and dissolve.
3. Measure 500 µl of the Tris-$CaCl_2$ Buffer (pH 8.0) into a centrifuge tube.
4. Add 20 mg Proteinase K.
5. Mix gently to dissolve the Proteinase K.
6. Sterilize the solution with a 0.22 µm filter.
7. Fill to a final volume of 1 ml with 100% glycerol.
8. Mix until completely dissolved.
9. Store in aliquots at -20°C for 1 year.

Note: Proteinase K interacts with nucleases. To ensure maximum Proteinase K activity, use water that has been treated with 0.1% DEPC to make the buffer. Add 1 ml of DEPC to 1 L of H_2O, shake vigorously, and let incubate for 12 hours at 37°C to inactivate any RNases. Autoclave the solution to inactivate DEPC before making Tris buffer.

1M Tris Buffer - 1 L

Instructions

1. Dissolve 121.14 g of Tris (Tris Base) in 750 mL of dH_2O.
2. Adjust to desired pH using concentrated HCl.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of Tris base and Tris HCl (Tris HCl, GoldBio Catalog # T-095) can be mixed to attain a pH of ~ 8.1. The pH can be adjusted by increasing the molar ratio of Tris HCl (more acidic) or Tris base (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M Tris, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.82
Adjust pH with: conc. HCl

pH	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
mL	77	75	73	72	70	67	64	61	57	53	49	45	41	37	32	28	25	21	18	15	12

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

Tris pKa at 25°: 8.06
Tris pH range: 7.0 – 9.0
d(pKa)/dT value: -0.028

10X TE Buffer – 1L

Instructions

1. Measure 100 ml of 1M Tris Buffer (See Tris Buffer Stock Solution protocol).
    a. **Tris Buffer can made to pH 8.0 for working with DNA or pH 7.5 for RNA.**

2. Add 20 ml of 0.5M EDTA Disodium

3. Fill to a final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at room temperature.

Note: A 1:10 dilution of TE Stock Solution with dH_2O will create a 1X working solution. 1X TE will contain 10mM Tris and 1mM EDTA.

Nucleic Acids Extraction - Nuclease Inactivation

Utilizing Proteinase K

Introduction

Proteinase K is used in the extraction of nucleic acids. After cell lysis, nucleases are released that degrade DNA and RNA. Proteinase K can effectively inactivate these nucleases by digesting them. Proteinase K can also help by digesting other proteins that are capable of contaminating your nucleic acid sample. Proteinase K can be used in most any DNA or RNA extraction protocol after cell lysis, but before extraction. The use of proteinase K is described below in a general nucleic acid extraction protocol.

Materials

- Proteinase K
- Any other materials that are used in your extraction protocol

Method

Cell Lysis

1. Lyse cells using your lysis method of choice.

Note: Proteinase K is compatible with guanidinium chloride, guanidinium thiocyanate, urea, iodoacetate, citrate, sodium dodecyl sulfate (SDS), Triton X-100, Tween 20 and EDTA.

Nuclease Digestion

2. Add Proteinase K to the lysate (See Proteinase K Stock Solution protocol).

Note: The final concentration of Proteinase K in solution should be 50-400 µg/ml.

3. Incubate sample at 55°C for 1- 3 hours.

Note: Samples may be digested overnight to be sure of complete nuclease digestion.

Note: Proteinase K can then be inactivated by heating to 95°C for 10 minutes after digestion.

Extraction

4. Extract the desired nucleic acid using your extraction method of choice.

DNA Extraction from Mouse Tail

Utilizing Proteinase K

Introduction

Clean genomic mouse DNA is used for the purposes of genotyping and molecular cloning. Mice are often used as a model organism to study mammals and the tail is a convenient place from which to extract DNA. Sodium Chloride and Sodium Dodecyl Sulfate are used to lyse the cells and Proteinase K serves to prevent degradation of genomic DNA during lysis by digesting nucleases. EDTA also helps to inactivate nucleases by chelating metal ions required correct conformation of the nucleases. Ethanol precipitation is a safe and easy method of isolating genomic DNA.

Materials

- Proteinase K
- 1M Tris Buffer (See Tris Buffer Stock Solution protocol)
- EDTA Disodium
- Sodium Chloride
- Sodium Dodecyl Sulfate (SDS)
- 100% Ethanol
- 70% Ethanol

Method

Stock Solution Preparation

1. Prepare Lysis Buffer stock solution (50 ml).
    a. Add 584 mg of NaCl.
    b. Add 93 mg of EDTA Disodium.
    c. Add 125 mg SDS.
    d. Add 5 ml of 1M Tris Buffer, pH 8.0 into a beaker.
    e. Fill to a final volume of 50 ml with pure H_2O.
    f. Store in aliquots at -20°C.

Sample preparation

1. Add 288 µl of Lysis Buffer to each 1.5 ml microcentrifuge tube.

2. Add 6 µl of thawed Proteinase K Stock Solution, 20 mg/ml (See Proteinase K Stock Solution protocol), to each 1.5 ml tube just before use.

Nuclease Digestion

1. Add a 2 mm length of mouse tail each 1.5 ml tube.

2. Incubate samples at 55°C overnight.

Precipitation

1. Add 1 ml 100% ethanol and mix well.

2. Spin samples with a centrifuge at 16,000 x g for 30 minutes.

3. Remove the supernatant.

4. Wash the pellet with 1 ml of 70% ethanol.

5. Spin the samples with a centrifuge at 16,000 x g for 20 minutes.

6. Remove the supernatant and immediately perform step 12.

Storage

1. Dissolve the DNA pellets in 300 µl of 1X TE buffer (See 10X TE Buffer Stock Solution protocol).

2. Place the samples on heat with the lids open at 55°C for ~2 hours or until all ethanol is evaporated.

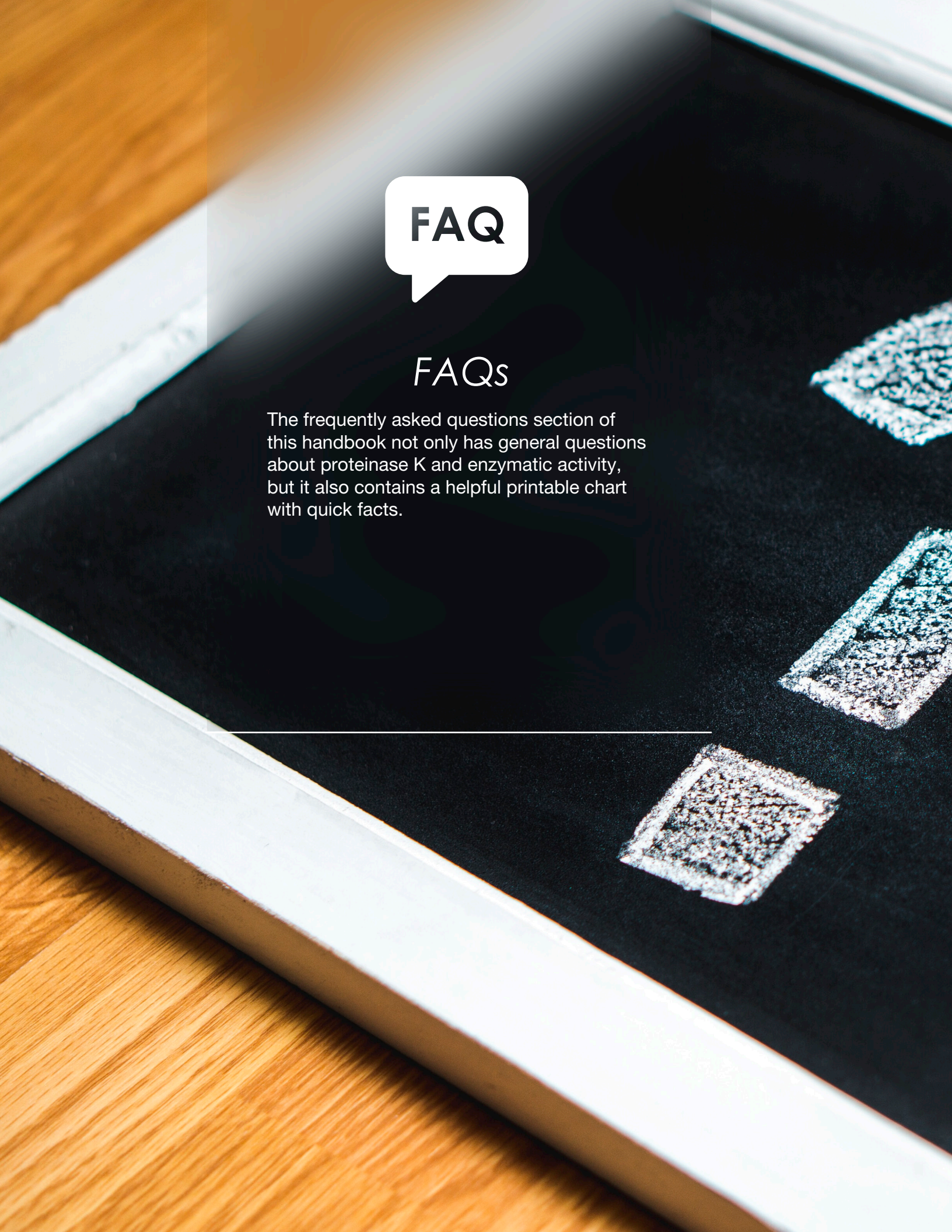
3. Store DNA samples at 4°C or -20°C and avoid freeze-thaw cycles.

Tips

- **WARNING: ethanol is flammable. Do not use near open flame.**

Associated Products

- Proteinase K (GoldBio Catalog # P-480)
- EDTA Disodium, dehydrate (GoldBio Catalog # E-210)
- Tris (Tris Base) (GoldBio Catalog # T-400)
- Tris HCl (GoldBio Catalog # T-095)



FAQs

The frequently asked questions section of this handbook not only has general questions about proteinase K and enzymatic activity, but it also contains a helpful printable chart with quick facts.

Answers to Important Proteinase K Questions

by Karen Martin & Adriana Richardson

A lot of questions pop up when it comes to using proteinase K, such as how to inactivate proteinase K, how should proteinase K be stored, what is the solubility of proteinase K… The list goes on. We decided to compile the most important questions and turn this page into the go-to resource for the product. Here is some important information about proteinase K.

How do you inactivate proteinase K?

Inactivating proteinase K is perhaps one of the most common questions we see. And the answer is very simple. Heat is a widely used way of inactivating proteinase K. While the activity of proteinase K increases with temperature, and is optimized at about 65 ˚C, heating proteinase K to 95˚C for 10 minutes 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.

Protease inhibitors such as PMSF and AEBSF (Pefabloc®) can also be used to permanently inactivate proteinase K.

Note- the actual inactivation temperature has been debated, ranging between 70 - 95˚C. However, crowd sourced feedback and extensive research led us to settle on 95˚C as the best temperature for inactivation.

What is the optimal temperature for proteinase K activation?

The optimal temperature for activity ranges between 37-65˚C, and depends on what tissue type you are working with. Higher temperatures help with protein unfolding, easing the ability for proteinase K to breakdown those proteins.

For FFPE tissue, digestion temperature range is 55-56°C. If you're working with a bacteria sample, digestion may vary between 37°C and 55-56°C.

Mammalian samples also vary, and much of the digestion temperature will depend on other factors, such as the incubation period. Experiments conducted with a digestion period overnight often cite 37°C, whereas shorter incubation times hold temperatures between 50-65°C.

But optimizing your proteinase K might not be the most important thing during your procedure. Sometimes, special techniques will require adapted temperatures to yield the best overall results. Therefore, something else to 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 65˚C, as temperatures increase, you risk inactivating proteinase K.

What is the relationship between proteinase K and calcium?

Proteinase K binds to two Ca^{2+} ions which helps 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 Ca^{2+}, 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 Ca^{2+}. Their findings suggest a method for treating contaminated RNase free DNaseI or isolating highly polymerized RNA.

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 (see #3). 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.

What are the activators of proteinase K?

Proteinase K activators include SDS (sodium dodecyl sulfate) and urea. Generally, proteinase K becomes more stable and more active when in buffers that contain these activators.

Proteinase K is also activated by heat at an optimal range of 50-65°C, dependent on your sample type.

How is proteinase K involved with cell lysis?

The first thing to consider is what proteinase K is. It's a broad-spectrum protease capable of digesting a wide range of native proteins (more details are later in this article).

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?

You want the RNase 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.

The answer to this question is really rooted in 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.

What applications is proteinase K used for?

Proteinase K is a broad-spectrum serine protease under the subtilisin-like class. As a broad-spectrum protease, it is primarily used for the isolation of nucleic acids: genomic DNA, cytoplasmic RNA, highly native DNA and RNA, etc. It is 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 used for:

- Digestion of unwanted proteins in molecular biology applications
- Removal of endotoxins bound to cationic proteins such as lysozyme and RNase A
- Removal of nucleases for *in situ* hybridization
- Prion research with respect to TSE (transmissible spongiform encephalopathies)
- Protease footprinting
- Mitochondrial isolation
- Isolation of genomic DNA
- Isolation of cytoplasmic RNA
- Isolation of highly native DNA or RNA

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

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.

How much solution would 1 gram of your proteinase K make?

Pack Sizes	Solution Equivalent
100 mg	5 ml (20 mg/ml)
500 mg	25 ml (20 mg/ml
1 g	50 ml (20 mg/ml

This is with an activity of 30 units/mg and 600 AU/ml in a 20 mg/ml solution.

What can proteinase K be dissolved in / How do you dissolve proteinase K?

Proteinase K is very water soluble, and it can also be dissolved in 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.

Is there an alternative to using proteinase K during DNA extractions?

The benefit of using proteinase K during DNA extraction is its ability to degrade a wide range of damaging nucleases. It's also great for digesting surface proteins on the cell membrane. However, 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

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 PrP^{SC} (disease causing isoform). Both PrP^{C} and PrP^{SC} have the same molecular weight; however, PrP^{SC} is resistant to proteinase K. Samples, which might contain both, are treated with proteinase K, which will eliminate PrP^{C} and convert PrP^{SC} into PrP^{RES} which has a lower molecular weight and can be pelleted, and therefore distinguished.

What is the optimal pH for proteinase K?

Proteinase K is active in a pH between 7.5 and 12.0.

What are Proteinase K inhibitors?

Proteinase K is inhibited by serine protease inhibitors such as PMSF, AEBSF, and DFP. Elevated temperatures may partially inactivate Proteinase K by heating at 95°C for 10 minutes. Keep in mind trace amounts of activity may still remain.

Proteinase K Quick Facts

General Info

Other Names	protease K, endopeptidase K, ProK
Protease Type	Serine protease of the subtilisin family
Molecular Weight	28.5 kDa
Activity	30 units/mg, 600 A U/ml in 20 mg/ml solution

Usage Info

Storage	-20 °C up to 1 year
Gram-Solution Conversion	(20 mg/ml sln) 1 gram produces 50 ml
Common Applications	DNA & RNA isolation, genomic DNA isolation, protein digestion
Solubility	Dissolves in water, tris & PBS

Activation & Optimization

Optimal Temperature	50 - 65 °C (active between 25 - 65 °C)
Optimal pH	7.5-12
Activators of Proteinase K	Ca^{2+}, SDS, Urea

Inactivation

Inactivation Temperature	95 °C for 10 minutes
Proteinase K Inhibitors	AEBSF (Pefabloc ®), PMSF, DIFP

GOLDBIO®

Enzyme Units FAQ

For proteinase K and other proteases

How does an enzyme unit describe activity?

An enzyme is usually valued for its function and activity rather than its mass, therefore enzyme units serve as a quantification of enzyme activity. For many enzymes, the activity under specified conditions can be expressed in *International Units* (IU), which is defined as the amount of enzyme that converts 1 µmol of a given substrate to a given product per minute.

What is specific activity?

Specific activity is defined as the enzyme activity per mass of protein and is seen as a measure of an enzyme's purity.

How are enzyme units defined for Proteinase K?

For enzymes that use large non-specific macromolecules as substrates (such as amylases or proteolytic enzymes), defining the molecular mass of the substrate can be tricky because in actuality the substrate changes each time a bond of the macromolecule is cleaved by the protease. Activity for these types of enzymes are usually determined by measuring the change in color intensity of the protein splits products per minute and using a standard curve to estimate the concentration of those products.

In 1938, M. L. Anson used denatured hemoglobin as a substrate for proteolytic enzymes in a Lowry assay. The undigested protein was then precipitated and the concentration of "protein split products" was estimated colorimetrically. Thus, the term Anson units was popularized and mAnson Unit was defined as the amount of enzyme that liberates 1 µmol of TCA-soluble, Folin-positive amino acids within 1 minute at pH 7.5 and 37°C, using hemoglobin as a substrate. In 1958, Hagihara decided to use casein as a substrate instead of hemoglobin. The term protease units is commonly used and can be defined in the same manner as mAnson units, except using casein as the substrate. mAnson units (that use hemoglobin as a substrate) are considered equivalent to protease units (that use casein as a substrate) when defining the activity of Proteinase K or other proteolytic enzymes.

What is a Folin-positive amino acid?

After digestion of a protein with Proteinase K, the protein is precipitated with TCA. The amino acids (protein split products) that remain in the solution are incubated with Folin-Ciocalteu reagent (FCR). FCR primarily reacts with tyrosine, but also tryptophan and cysteine so each of these amino acids would be considered Folin-positive. A standard curve is generated using tyrosine and the concentration of TCA-soluble, Folin-positive amino acids is estimated by comparing the color intensity of the protein split products with the color intensity of the tyrosine standards.

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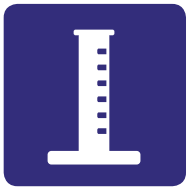

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