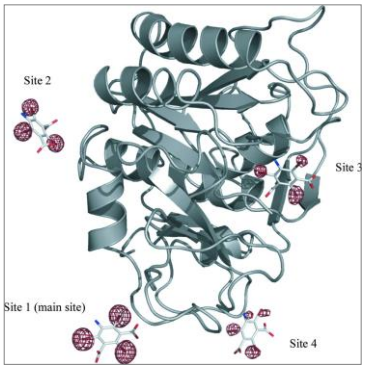
FT-718961

Proteinase K, solution

For nucleic acid extraction protocols but also for protein fingerprinting experiments, and for removal of nucleases.

Product Description

Name :	**Proteinase K, Solution, 20 mg/ml** **from *Tritirachium album timber*** Syn.: peptidase K, Tritirachium alkaline proteinase
Catalog Number :	718962L, 1.5ml 718961, 10ml 0
Structure :	MW: 29 730KDa E.C.: 3.4.21.14 CAS: [39450-01-6] EINECS: 2544578
Specific Activity :	>30 Units/mg
Form :	20mg/mL proteinase K solution
Technical Data :	
Storage:	Stable at +2 to +4°C for long term, but can also be stored at Room Temperature(L) Dissolve then aliquot and store at -20ºC recommended. See also item 718961 (M).



B3C in proteinase K
(Biological Crystallography, 66:Part 4:374-380, 2010)

Introduction

Proteinase K (PK) is a broad substrate non-specific serine proteinase. It is very stable at pH 4-12. It is used on isolating mRNA, genomic DNA and digesting unwanted proteins during DNA and RNA preparations from different kinds of cells. It's been used on glycoprotein modification and protein structure studies also. Proteinase K is active with SDS, urea and EDTA.

Proteinase K is used for the isolation of native high molecular genomic nucleic acids. Enzymes like DNases and RNases from microorganisms and mammalian cells are rapidly inactivated by Proteinase K.

Adding Proteinase K already during the cell lysis enables the isolation of highly native undamaged high molecular DNA or RNA. A variety of methods have been established, which are documented in numerous publications. Recently, Proteinase K has been used for the detection of BSE forming proteins which are uniquely resistant towards the enzyme's proteolytic cleavage.

Proteinase K is very useful in the analysis of membrane structure by means of modification of proteins and glycoproteins on cell surfaces.

Because of the cleavage specificity Proteinase K, characteristic fragments of proteins are obtained which are helpful in revealing the structure and function of proteins, particularly enzymes.

Directions for use

DNA Isolation from Tails:

1. Each tail should be in a clean eppendorf tube.
2. Add 500µl of tail lysis buffer containing Proteinase K (PK) to each tube.
3. Incubate tail samples in 50-60°C water bath overnight.
4. Add 250µl saturated (6M) NaCl to each tube.
5. Shake tubes vigorously (~ 20 times) and incubate tubes on ice for 10 minutes.
6. Spin tubes on low speed (#6 on Hemle centrifuge) at 4°C for 10 minutes.
7. Remove supernatant and place into a clean eppendorf.
8. Add 650µl isopropanol and invert to mix. Incubate tubes at room temperature for 15 minutes.
9. Recover DNA by centrifuging, max speed, 10 minutes at room temp.
10. Place tubes inverted on bench and allow to air dry 5 minutes.
11. Add 200µl of TE pH 7.5 or sterile water to each tube. Incubate in 50-60°C water bath for 10 minutes. Resuspend pellet by pipetting up and down several times.

Tail Lysis Buffer:

	Final Concentration	Per 500 ml
1M Tris pH 8,0	10mM	5ml
5M NaCl	100mM	10ml
0,5M EDTA pH 8,0	10mM	10ml
10% SDS	0,5%	25ml
dH_2O		To 500ml

Proteinase K concentration:

Add 20µl of a 20 mg/ml stock per 1ml of tail lysis buffer.

Embryonic stem cell (ES Cells):

For ES Cells the protocol is very much the same except for the following:
All steps are done in a well of a 24 or 6-well dish.
The initial incubation in the lysis buffer is done at 37°C for 2 hours to overnight.

Southerns: For important Southerns:

1. Dilute DNA in 400µl of water.
2. Phenol/chloroform extract DNA.
3. Precipitate in 1/10 vol 3M NaOAc and equal volume of isopropanol.
4. Precipitate 15 minutes at RT.
5. Wash pellet with 70% EtOH.
6. Resuspend in water.

Proteinase K Antigen Retrieval Protocol

Description: Formalin or other aldehyde fixation forms protein cross-links that mask the antigenic sites in tissue specimens, thereby giving weak or false negative staining for immunohistochemical detection of certain proteins. The Proteinase K based solution is designed to break the protein cross-links, therefore unmask the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thus enhancing staining intensity of antibodies.

Solutions and Reagents: Proteinase K Solution (Method 1) (20 µg/ml in TE Buffer, pH 8.0):

TE Buffer (50mM Tris Base, 1mM EDTA, 0.5% Triton X-100, pH 8.0):

Tris Base ---------- 6.10 g
EDTA ---------- 0.37 g
Triton X-100 ---------- 5 ml
Distilled water ---------- 1000 ml

Mix to dissolve. Adjust pH 8.0 using concentrated HCl (10N HCl). Store at room temperature.

Proteinase K Stock Solution (20x, 400 µg/ml or 12 units/ml):

Proteinase K (30 units/mg)---------- 0.008 g (8 mg)
TE Buffer, pH8.0 ---------- 10 ml
Glycerol ---------- 10 ml

Add Proteinase K to TE buffer until dissolved.
Then add glycerol and mix well. Aliquot and store at –20ºC for 2-3 years.

Working Solution (1x, 20 µg/ml or 0.6 units/ml):

Proteinase K Stock Solution (20x) ------ 1 ml
TE Buffer, pH8.0 ---------- 19 ml

Mix well. This solution is stable for 6 month at 4 ºC.

References

Bogard R. *et al*., MetR-Regulated Vibrio cholerae Metabolism Is Required for Virulence, *mBio*, 3: e00236-12 (2012) Article

Eskeland R *et al*., HP1 Binding to Chromatin Methylated at H3K9 Is Enhanced by Auxiliary Factors, *Mol. Cell. Biol.*, 27: 453 - 465 (2007) Article

Legals

Hazard Statements: H315 / H317 / H319 / H334 / H335
Precautionary Statements: P280 / P302+P352 / P304+P340 / P305+P351+P338
Hazard Code: ghs08 UN Number: NONE

Related / associated products and documents

- TRIS HCl, UP09154E
- EDTA, UP036290
- Tris-EDTA buffer, pH 8, 587528
- Sodium Chloride, 89678A
- Nonidet P-40, WZ7550
- Ribonuclease A (RNAse A), 91842A

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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