FT-718961

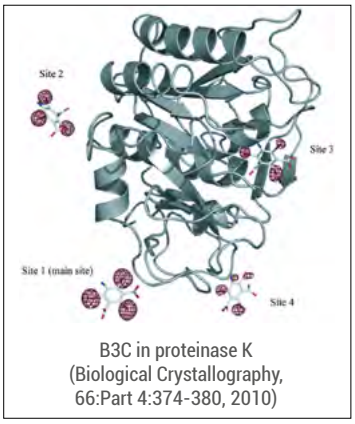
Uptima

# 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, 20mg/mL**<br>from *Tritirachium album*<br>Syn.: peptidase K, Tritirachium alkaline proteinase |
| **Catalog Number:** | **718962**, 1mL<br>**718961**, 5x1mL |
| **Structure & Properties:** | MW: ~27KDa E.C.: 3.4.21.14<br>CAS: [ 39450-01-6 ] EINECS: 2544578 |
| **Specific Activity:** | >30 Units/mg |
| **Form:** | 20mg/mL proteinase K, 1mM $CaCl_2.(H_2O)_2$; 10mM Tris-HCl (pH 7.5); 30% glycerin. |
| **Technical Data:** | Activity: min. 600mAnsonU/mL (>30mAU/mg)<br>[ One Anson Unit (AnsonU) is defined as the amount of enzyme that liberates Folin-positive amino acids and peptides, corresponding to 1 µmol tyrosine under assay conditions in 1 minute using haemoglobin as substrate (Anson M.M, J. Gen. Physiol., 22 : 79, 1939) ] |
| **Storage:** | Stable at -20°C for 2 years for long term, but can also be stored at 4°C(M)<br>See also item 71896L for a more stable formulation, even at RT(L). |




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

## Introduction

Proteinase K (CAS: 39450-01-6) is a non-specific serine protease having a very high specific activity (cleaves the carboxylic ends of aromatic, hydrophobic and aliphatic amino acids). It has been used for isolation of mRNA, high molecular weight DNA and to inactivate other enzymatic activities. Proteinase K is active with or without the presence of SDS and EDTA.
The cleavage range is very broad: Proteinase K cleaves the carboxylic ends of aromatic, hydrophobic and aliphatic amino acids, making it useful for general digestion of proteins in biological samples (Ebeling W. et al. (1974) Eur. J. Biochem., 47, 91). Proteinase K is mainly used in nucleic acid extraction protocols but may also be used in protein fingerprinting experiments, or for removal of nucleases.

Proteinase K is extracted from the fungus *Tritirachium album*

### Quality Control

| | |
|---|---|
| 16-hour incubation: | a 50µL reaction solution containing 1µg of lambda-DNA and 1.8 units enzyme incubated for 16 hours at 37°C resulted in the same DNA band pattern after gel electrophoresis as compared to the pattern produced without enzyme. |
| Exonuclease activity: | Incubation of 6 units of the enzyme for 4 hours at 37°C in 50µL assay buffer with 1g sonicated 3H DNA (3 x 10(5) cpm/µg) released less than 0.2% of radioactivity. |
| Endonuclease activity: | Incubation of 1.8 units of enzyme with 1µg PhiX174 RFI DNA in 50µL assay buffer for 4 hours at 37°C gave less than 1.5% conversion of RFI. |
| RNAse contamination: | Incubation of 6 units of enzyme with 1 µg MS2 RNA in 50µL assay buffer for 4 hours at 37°C resulted in the same RNA band pattern after gel electrophoresis as compared to the pattern produced without the enzyme. |

## 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 500mL |
|---|---|---|
| 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) (20µg/mL in TE Buffer, pH 8.0):
TE Buffer (50mM Tris Base, 1mM EDTA, 0.5% Triton X-100, pH 8.0):
Tris Base ---------- 6.10g
EDTA---------- 0.37g
Triton X-100 ---------- 5mL
Distilled water ---------- 1000mL
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) 0.008g (8mg)
TE Buffer, pH8.0 ----------10mL
Glycerol----------10mL
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, 20µg/mL or 0.6 units/mL):
Proteinase K Stock Solution (20x) -- 1mL
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 4 70 03 73 06