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 from *Tritirachium album*  
| Syn.: peptidase K, *Tritirachium* alkaline proteinase |
| Catalog Number: | 718962, 1mL  
| 718961, 5x1mL |
| Structure & Properties: | MW: ~27KDa  
| E.C.: 3.4.21.14 |
| Specific Activity: | >30 Units/mg |
| Form: | 20mg/mL proteinase K, 1mM CaCl₂(H₂O)₂; 10mM Tris-HCl (pH 7.5); 30% glycerin. |
| Technical Data: | Activity: min. 600mAnsonU/mL (>30mAU/mg)  
| [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)  
| See also item 71896L for a more stable formulation, even at RT (L). |

**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.

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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 -20°C 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°C.
References


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

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