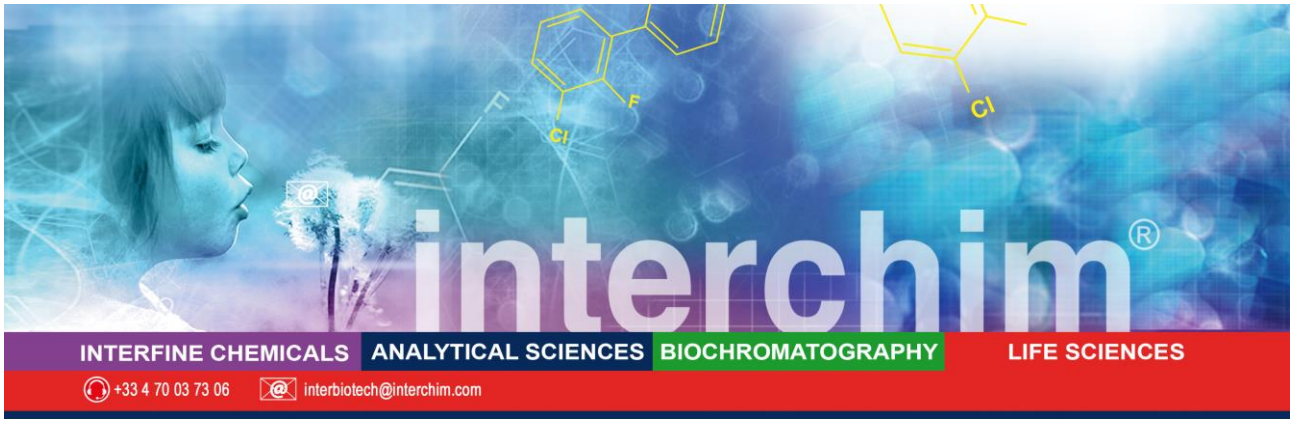
FT-858707

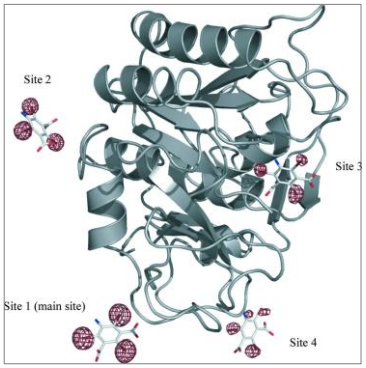


Proteinase K

For nucleic acid extraction protocols but may also be used in protein fingerprinting experiments, or for removal of nucleases.

Product Description

Name :	**Proteinase K** from *Tritirachium album*
Catalog Number :	858707 , 100mg 858709 , 500 mg 858708 , 1g
Structure :	CAS: 39450-01-6 MW: 28 500
Specific Activity :	>30 Units/mg
Unit Definition :	One unit is defined as the amount of enzyme that liberates 1.0 µmol tyrosine from casein per miute at 37°C, pH 7,5.
Technical Data :	Activity >30 U/mg Specific Activity >40 U/mgP
Storage:	Stable at +4°C



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

Proteinase K 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.

Directions for use:

- DNA Isolation from Tails
- Embryonic stem cell (ES Cells)
- Southerns
- Proteinase K Antigen Retrieval

Directions for use

DNA Isolation from Tails:

- Each tail should be in a clean eppendorf tube.
- Add 500µl of tail lysis buffer containing Proteinase K (PK) to each tube.
- Incubate tail samples in 50-60°C water bath overnight.
- Add 250µl saturated (6M) NaCl to each tube.
- Shake tubes vigorously (~ 20 times) and incubate tubes on ice for 10 minutes.
- Spin tubes on low speed (#6 on Hemle centrifuge) at 4°C for 10 minutes.
- Remove supernatant and place into a clean eppendorf.
- Add 650µl isopropanol and invert to mix. Incubate tubes at room temperature for 15 minutes.
- Recover DNA by centrifuging, max speed, 10 minutes at room temp.
- Place tubes inverted on bench and allow to air dry 5 minutes.
- 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 Proteinase K stock solution 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:

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

Related / associated products and documents

Technical notice on Proteinase K (NT-85870n)

- 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

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