

## Manual


# Proteinase K (lyophilisate)

Enzyme for digesting proteins in biological samples. Activity  $\geq 30$  U/mg.

REF	size
1019IV-25L	25 mg
1019IV-100L	100 mg
1019IV-250L	250 mg
1019IV-500L	500 mg
1019IV-B1L	1 g
1019IV-B50L	50 g
1019IV-B100L	100 g
1019IV-150U	150 U
1019IV-750U	750 U

For in vitro diagnostics use.



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### Guarantee

A&A Biotechnology provides guarantee on this product.

The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components



## Description

**Proteinase K** (EC 3.4.21.64) is a broad-spectrum serine protease. The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups. Proteinase K is inactivated by diisopropyl fluorophosphate (DFP) or phenyl methane sulfonyl fluoride (PMSF). It's a recombinant enzyme cloned from fungus *Engyodontium album* and produced in *Pichia pastoris*.

## Application

- extremely effective proteolytic degradation of biological material
- inactivation of endogenous DNases and RNases during nucleic acid purification

## Contents

	1019IV-25L	1019IV-100L	1019IV-250L	1019IV-500L	storage
proteinase K, recombinant, lyophilisate	25 mg	100 mg	250 mg	500 mg	2-8 °C*
	1019IV-B1L	1019IV-B50L	1019IV-B100L		
proteinase K, recombinant, lyophilisate	1 g	50 g	100 g		2-8 °C*
	1019IV-150U	1019IV-750U			
proteinase K, recombinant, lyophilisate	150 U	750 U			2-8 °C*

\* Transport at 15-25 °C.

## Unit definition

One unit of proteinase K hydrolyzes denatured hemoglobin to produce color equivalent to 1.0 µmol of tyrosine per minute at pH 7.5 and 37 °C (color development with Folin & Ciocalteu's Phenol Reagent).

## References

1. Burkiewicz A., Dąbrowski S., Barski P., Polska rekombinowana Proteinaza K, (2007) *Postępy Biochemii* 53(4): 327-328
2. Ebeling W., Hennrich N., Klockow M., Metz H., Orth H.D., Lang H., (1974) *Eur. J. Biochem.* 47(1):91-97
3. Hilz H., Wiegiers U., Adamietz P., (1975) *Eur. J. Biochem.* 56(1):03-08
4. Betzel C., Sigh T.P., Visanji M., et al., (July 1993) *J. Biol. Chem.* 268(21):15854-15858

# Protocol

To obtain proteinase K solution add appropriate amount of sterile water (not included) or recommended storage buffer: 20 mM Tris, pH 7.5, 1 mM CaCl<sub>2</sub>, 0.02% sodium azide, 50% glycerol (v/v) (not included) and dissolve whole content. For long-term storage of the solution, we recommend dissolving the lyophilizate in buffer (20 mM Tris, pH 7.5, 1 mM CaCl<sub>2</sub>, 0.02% sodium azide, 50% glycerol (v/v))

Store prepared solution at 2–8 °C.

**Note:** Proteinase K undergoes natural autoprolysis at concentrations below 1 mg/ml; this should be taken into account when preparing the solution.

## Safety information

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**DANGER**

### Proteinase K

H315 Causes skin irritation.  
H319 Causes serious eye irritation.  
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.  
H335 May cause respiratory irritation.  
P261 Avoid breathing dust.  
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.  
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor/physician.









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# Troubleshooting

Problem	Possible cause	Suggested solution
<b>Powder dissolves poorly / solution is cloudy</b>	Target enzyme concentration is too high.	Lower the target enzyme concentration. If the solution becomes cloudy, centrifuge and use the clear supernatant.
	Inappropriate buffer or water used for solution preparation.	Use the recommended buffer/ultrapure water.
	Insufficient or overly vigorous mixing.	Apply appropriate mixing (e.g., tube inversion, mixing on a rotator).
	Partial degradation of an old or improperly stored lyophilisate.	Use a fresh enzyme batch.
<b>Low or no activity after dissolution</b>	Incorrect pH or buffer composition (e.g., lack of Ca <sup>2+</sup> ).	Check the pH and buffer composition.
	Storage of the solution at too high a temperature.	Prepare a fresh solution from the lyophilisate.
	Repeated freezing–thawing cycles.	Prepare small aliquots and avoid refreezing.
	Presence of protease inhibitors.	Ensure that no inhibitors are present in the mix.
	Error in concentration calculation or dosing.	Verify enzyme calculations and dosing. Perform an activity test on a control sample.
<b>Gradual loss of activity during solution storage</b>	Storage time too long or at an incorrect temperature.	Follow the recommended temperature and storage time.
	No aliquoting and repeated sampling from a single tube.	Store the enzyme in small aliquots.
	Protein adsorption to tube walls.	Use appropriate tubes (e.g., PP).
	Slow degradation due to improper pH or contaminants.	Use the recommended buffers/stabilizers. If in doubt, prepare a fresh solution.
	Autoproteolysis.	Prepare the solution at a concentration above 1 mg/ml.
<b>Suspected microbiological contamination of the solution</b>	Lack of aseptic technique during preparation.	Prepare the solution under aseptic conditions. Use sterile tubes and pipette tips.
	Prolonged storage of the solution at a temperature higher than recommended.	Store the solution at the recommended temperature.

Problem	Possible cause	Suggested solution
Lack of the expected effect in the procedure (e.g., DNA/RNA isolation)	No sterile filtration before planned long-term storage.	After reconstitution, filter the solution. If visible contamination is present, discard the solution and prepare a new one from the lyophilizate
	Enzyme concentration too low or too high.	Verify the recommendations of the kit/method manufacturer and adjust the amount of enzyme.
	Incorrect incubation time or temperature.	Adjust the incubation time and temperature.
	Sample-specific factors.	Modify sample preparation (e.g., more thorough homogenization, buffer change). Perform the reaction on a reference sample as a control.

## Explanation of symbols used

symbol	symbol meaning	symbol	symbol meaning
	Indicates the <i>In vitro</i> diagnostics medical device		Indicates the manufacturer's catalogue number
	Indicates the medical device manufacturer		Indicates the need for the user to consult the instructions for use
	Indicates the manufacturer's batch code or lot can be identified		Indicates the need for the user to consult the instructions for use for important information such as warnings and cautions
	Indicates the date after which the medical device is not to be used		Indicates the temperature limits to which the medical device can be safely exposed





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