

EcoZyme Proteinase K Troubleshooting Guide

1 mL, 5 x 1 mL

Cat No: EZPK1, EZPK5

Use this guide for common protein-digestion and handling problems encountered with EcoZyme Proteinase K solution. The product is supplied as a 20 mg/mL molecular biology grade solution and is intended for general protein digestion and nucleic-acid preparation workflows.

Before You Start - Quick Checks

1. The ready-to-use solution is supplied at 20 mg/mL in 10 mM Tris-HCl (pH 7.5), 1 mM CaCl₂, and 50% glycerol.
2. Store at -20°C.
3. For supported digestion conditions, the datasheet states use at 56°C for up to 4 hours or at 37°C for overnight incubations.
4. Proteinase K is a nonspecific serine protease used for protein digestion and for inactivation of RNases and DNases in nucleic-acid preparation workflows.
5. Use clean nuclease-free labware when the treated material will be used for nucleic-acid applications.

Problem	Most likely cause	Recommended action
Protein digestion is incomplete	The incubation time or temperature may be insufficient for the sample.	Use the supported conditions in the datasheet: up to 4 hours at 56°C or an overnight incubation at 37°C, as appropriate for the workflow.
Digestion varies between samples	Different sample loads or matrix complexity may require different exposure to Proteinase K.	Keep sample input and enzyme addition consistent and use the same validated incubation condition across comparable samples.
Low performance after storage	The enzyme may not have been maintained at the specified storage temperature.	Store the product at -20°C and compare with a properly stored aliquot if storage history is uncertain.
High-molecular-weight nucleic acid preparation is inconsistent	Protein digestion or nuclease inactivation may be incomplete.	Ensure adequate Proteinase K treatment before proceeding to nucleic-acid purification and use one of the supported incubation conditions.
Residual protein remains after treatment	Protein digestion may not have reached completion for the sample type.	Extend incubation within the supported time/temperature range or optimize the amount used in the validated workflow.
Unexpected nuclease-related degradation persists	The workflow may not have provided sufficient Proteinase K exposure for nuclease inactivation.	Confirm the enzyme was active and that digestion conditions were sufficient before downstream nucleic-acid handling.
Results differ after changing incubation temperature	The kinetics of digestion differ between 56°C and 37°C conditions.	Do not compare the two conditions directly without validation; use a single optimized temperature/time combination for a given workflow.
Enzyme solution appears nonuniform after thawing	The viscous glycerol-containing solution may not have been sampled consistently.	Allow the solution to thaw sufficiently for accurate pipetting and mix gently until uniform before dispensing.
Unexpected downstream inhibition	Excess treatment solution or incomplete cleanup may be carried into the next step.	Use the enzyme amount specified in the validated workflow and complete the intended downstream purification or cleanup step before sensitive applications.

Still having trouble? Verify -20°C storage, confirm that the 20 mg/mL solution was added accurately, and use a validated digestion condition within the supported 56°C/up to 4 h or 37°C/overnight range. If downstream nucleic-acid quality remains poor, recheck the complete sample-preparation workflow.