

EcoZyme Deoxyribonuclease I (DNase I)

Troubleshooting Guide

10 mg, 25 mg, 100 mg

Cat No: DI010, DI025, DI100

Use this guide for common activity, handling, and DNA-removal problems encountered with EcoZyme Deoxyribonuclease I (DNase I). The enzyme requires divalent metal ions for activity and is sensitive to physical denaturation and inhibitory reaction components.

Before You Start - Quick Checks

1. For short-term use store at 2-8°C; for long-term storage and after reconstitution store at -20°C and avoid repeated freeze-thaw cycles.
2. Reconstitute in EcoZyme DNase I Storage Buffer to 10 mg/mL, prepare aliquots, mix gently by inversion, and do not vortex.
3. DNase I activity requires divalent ions; EDTA and SDS inhibit the enzyme. Ca²⁺ helps stabilize the enzyme.
4. For RNA cleanup, use up to 50 µg total RNA with 10 units DNase I and 20 units ribonuclease inhibitor in a 50 µL reaction.
5. Incubate RNA-cleanup reactions at 37°C for 20-30 minutes.
6. Inactivate DNase I appropriately after treatment to avoid residual activity in downstream steps.

Problem	Most likely cause	Recommended action
Residual DNA remains after treatment	The reaction may contain inhibitors, insufficient active enzyme, or more RNA/sample material than the recommended setup.	Check for EDTA or SDS, use the recommended reaction buffer, and for RNA cleanup keep the reaction within the stated limit of up to 50 µg total RNA with 10 units DNase I.
Low or no DNase activity	Required divalent ions may be unavailable or chelated, or the enzyme may have lost activity during handling.	Use the recommended reaction buffer, avoid chelating agents such as EDTA during digestion, and verify that the enzyme was stored and handled as specified.
Activity varies between aliquots	The reconstituted enzyme may not have been mixed gently and uniformly or may have undergone repeated freeze-thaw cycles.	Reconstitute to 10 mg/mL, mix by gentle inversion, prepare single-use aliquots, and store at -20°C.
Activity decreases after mixing	DNase I is sensitive to physical denaturation and may have been vortexed or handled harshly.	Do not vortex. Mix by gentle inversion and briefly collect liquid from tube walls if needed.
RNA integrity decreases during DNase treatment	The RNA-cleanup reaction may not contain the recommended ribonuclease inhibitor or RNase-free handling may have been inadequate.	Include 20 units of ribonuclease inhibitor per 50 µL cleanup reaction as specified and maintain RNase-free technique.
Reaction is slower than expected	The incubation time, temperature, or reaction chemistry may not match the recommended conditions.	For the RNA-cleanup protocol, incubate at 37°C for 20-30 minutes and use the recommended buffer composition.
Downstream reaction is affected after DNase treatment	Residual DNase activity may remain because the enzyme was not inactivated or removed after digestion.	After treatment, use an appropriate inactivation method such as heat inactivation, EDTA addition, or RNA purification, according to the downstream application.
Reconstituted enzyme performance is inconsistent	The concentration may be incorrect or the enzyme may have been stored improperly after reconstitution.	Prepare the enzyme at the recommended 10 mg/mL concentration, aliquot it, store at -20°C, and avoid repeated thawing.
Unexpected inhibition in a complex sample	Sample components may interfere with DNase I activity.	Check the sample for EDTA, SDS, or other incompatible components and, where appropriate, purify or exchange the sample into a compatible reaction environment before digestion.

Still having trouble? Recheck storage and reconstitution history, confirm the absence of EDTA/SDS during digestion, verify the recommended enzyme and buffer amounts, and include ribonuclease inhibitor when treating RNA. If residual DNase activity is a concern, confirm that the post-treatment inactivation or purification step was completed.