Immobilized DNase I

For removal of DNA from samples

(Cat. # 786-1831)

INTRODUCTION

Immobilized DNase I is designed for applications where removal of DNA is required without contaminating the sample with DNases. For example, it is used in molecular biology for removal of DNA from samples for RT-PCR application. Some RT-PCR reactions require removal of DNA from samples to prevent their interference in RT-PCR reactions. Samples can be directly treated with DNase I, however this adds additional step of removing DNase I. Immobilized DNase I can also be used in cell free transcription and translation system such as derived from *E. coli*, where the endogenous DNA can be removed by treating the *E. coli* derived cell free system with immobilized DNase I agarose beads[1]. The immobilized DNase I agarose beads can also be used for purifying proteins that binds to it such as actin[2].

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1831	Immobilized DNase I	2 ml resin

STORAGE CONDITIONS

Immobilized DNase I is shipped with blue ice. Store the beads at -20°C upon receipt.

SPECIFICATIONS

- **Matrix**: 6% cross-linked Agarose.
- Coupling is covalent through linkage of amino group of the DNase I to the pre-activated agarose.
- **Extent of labeling**: 0.8-1.2 mg (1600-2400 units) DNase I per ml resin
- **Storage buffer**: 50% Glycerol PBS
- **Storage Temperature**: -20°C, avoid repeated thaw cycles, store in aliquots

IMPORTANT INFORMATION

1. Immobilized DNase I loses its activity if stored for long at 4°C. Store the beads in storage buffer at -20°C. Avoid repeated freeze-thaw of the beads.
2. The reaction buffer for removing DNA can be same as used for DNase I treatment of samples. For example, **1X Reaction buffer**: 10mM Tris-HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH7.5. Other buffers can be used, the key requirement is to have 2.5 mM $MgCl_2$ and 0.5 to 1 mM $CaCl_2$.
3. The maximum volume of Immobilized DNase I beads to reaction that can be used is 1:1. For example 50 µl of packed beads can be used to treat as low as 50µl of sample volume for removal of DNA. Volume of beads to sample volume and incubation times should be optimized by end user.

ADDITIONAL ITEMS REQUIRED

- Microfuge tubes
- Reaction buffer/wash buffer.

- **Storage buffer for beads**: 50% Glycerol PBS
- Sample

PROTOCOL

General guidelines for setting reaction for removal of DNA from samples

1. Take 20 to 100 µl Immobilized DNase I agarose beads in a microfuge tube.
   NOTE: *Volume of beads used in reaction needs to be optimized and determined by end user for desired results.*
2. Spin the tubes at 1000 g for 5 minutes at 4°C.
3. Remove the supernatant. Add 1 ml reaction buffer (1X) as wash buffer to the beads.
4. Resuspend the beads in the 1 x reaction buffer followed by centrifugation at 1000 g for 5 minutes at 4°C.
5. Discard the supernatant.
6. Add the test sample to beads, add the reaction buffer to the sample such that final concentration of sample has 10 to 50 mM Tris, 2.5 mM $MgCl_2$ and 0.5 to 1 mM $CaCl_2$.
7. Incubate the microfuge tube at 37°C on a shaker for 30 minutes to 2 hours depending upon requirement.
   NOTE: *Optimize the reaction time as per requirement.*
8. After the reaction, centrifuge the tubes at 1000 g for 5 minutes. Remove the supernatant/sample free of DNA and store or use for downstream applications.

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
DNA in the sample is not getting digested or degraded	Immobilized DNase I agarose beads were not stored properly	Store the beads in storage buffer at -20°C. Use properly stored fresh lot of DNase I agarose beads.
	Immobilized DNase I agarose beads used repeatedly and lost activity with multiple use	Use fresh unused Immobilized DNase I agarose beads.
	Immobilized DNase I agarose beads volume to sample volume not optimized	Increase the volume of beads used for the reaction
	Reaction time not optimized	Increase the time of incubation with beads to achieve desired results

REFERENCES

1. Moorman A. F. M. et al (1976). A Coupled transcription-translation system derived *Escherichia coli*: The use of immobilized deoxyribonuclease to eliminate endogenous DNA. FEBS letters 71: 67-72.
2. Andersland J. M. et al (1992). The isolation of actin from pea roots by DNase I affinity chromatography. Plant Physiology 100 (4): 1716-23.

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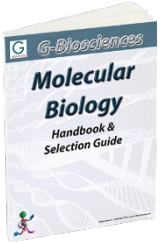

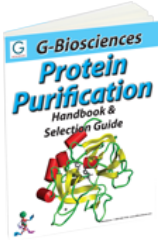

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