Protocol

TD-P Revision 2.1

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Nucleic Acids Extraction - Nuclease Inactivation

Utilizing Proteinase K

Introduction

Proteinase K is used in the extraction of nucleic acids. After cell lysis, nucleases are released that degrade DNA and RNA. Proteinase K can effectively inactivate these nucleases by digesting them. Proteinase K can also help by digesting other proteins that are capable of contaminating your nucleic acid sample. Proteinase K can be used in most any DNA or RNA extraction protocol after cell lysis, but before extraction. The use of proteinase K is described below in a general nucleic acid extraction protocol.

Materials

- Proteinase K (Proteinase K, GoldBio Catalog # P-480 [CAS 39450-01-6, mw.=28.9 kDa])
- Any other materials that are used in your extraction protocol

Method

Cell Lysis

1. Lyse cells using your lysis method of choice.

> **Note: Proteinase K is compatible with guanidinium chloride, guanidinium thiocyanate, urea, iodoacetate, citrate, sodium dodecyl sulfate (SDS), Triton X-100, Tween 20 and EDTA.**

Nuclease Digestion

2. Add Proteinase K to the lysate (See Proteinase K Stock Solution protocol).

> **Note: The final concentration of Proteinase K in solution should be 50-400 µg/ml.**

3. Incubate sample at 55°C for 1- 3 hours.

> **Note: Samples may be digested overnight to be sure of complete nuclease digestion.**

> **Note: Proteinase K can then be inactivated by heating to 95°C for 10 minutes after digestion.**

Extraction

4. Extract the desired nucleic acid using your extraction method of choice.

References

Gold Biotechnology
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