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Uracil-DNA Excision Mix

Cat. No. UEM04100

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1. Introduction

Uracil-DNA Excision Mix* contains two enzymes, HK™-UNG (Heat-Killable Uracil N-Glycosylase [UNG]) and Endonuclease IV. HK-UNG cleaves the uracil base from a uracil-deoxynucleotide in any DNA, creating an abasic site at the location of dUTP incorporation. Endonuclease IV then cleaves the phosphodiester bond at this abasic site generating a series of digestion fragments. The size of the fragments defines the presence and location of the uracil residues in the treated DNA.

Uracil-DNA Excision Mix (100 µl) is provided with 100 µl of a 10X Uracil Excision Enzyme Buffer.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Uracil Excision Enzyme 10X Buffer: is 500 mM Tris-HCl (pH 9.0), 200 mM $[NH_4]_2SO_4$ and 100 mM EDTA.

Activity Assay: Uracil Excision Enzyme Mix is function-tested in an assay by incubating DNA containing at least one uracil residue, in a standard reaction and analyzing the digestion products by polyacrylamide gel electrophoresis.

Contaminating Activity Assays: The Uracil Excision Enzyme Mix is free of detectable non-specific DNA exonuclease and endonuclease, and RNase activities.

3. Related Products

The following products are also available:

- MasterPure™ Nucleic Acid Purification Kits
- BuccalAmp™ DNA Extraction Kits
- dNTP Solutions
- dUTP Solution

4. General Considerations for Optimization of Excision/Cleavage Reactions

1. **Amount of DNA:** The optimal amount of DNA to be digested in the excision reaction depends upon the specific activity of the labeled primer and the length and its nucleotide composition. Excision/cleavage reactions containing DNA labeled with fluorescent dyes or nonradioactive molecules require more product for adequate detection (2-3 pmol, see below). Likewise, target sequences composed of a large percentage of A+T will require more DNA in the reaction.
2. **Excision/cleavage product concentration:** If necessary, concentrate the excision/cleavage reaction products by ethanol precipitation before loading the samples on a polyacrylamide gel.

** U.S. Patent No. 6,190,865.*

Excision/Cleavage of DNA Containing dUTP

DNA containing uracil residues is cleaved using the Uracil-DNA Excision Mix and then subsequently subjected to denaturing gel electrophoresis.

A. Assembly of the Excision/Cleavage Reaction

The volumes listed below are for a single excision reaction containing a total reaction volume of 10 µl. This volume may be increased or decreased by proportionally adjusting the individual reaction components.

1. Thaw and thoroughly mix all of the reagents before dispensing; keep on ice.

   Combine the following reagents, on ice:

x	µl	deionized water
2.5-5	µl	DNA containing dUTP (0.75-3 pmol)
1	µl	10X Uracil Excision Enzyme Buffer
1	µl	Uracil-DNA Excision Mix
10	µl	Total reaction volume

2. Incubate for 30 minutes at 37°C.
3. Add 5 µl of a Stop/Loading Buffer (ie. 95% formamide, 10 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue [final pH 9.5]) to each reaction; adjust the volume proportionally if the total reaction volume is different from 10 µl.

   Note: *Xylene cyanol and bromophenol blue may interfere with some fluorescence detectors. When using such detectors, use a stop/loading buffer containing 95% formamide that is compatible with the detection system (alternative dye).*
4. Cleavage products may be stored at –20°C for up to two weeks.

B. Typical Gel Electrophoresis Procedure

1. Prepare a standard 6-8% polyacrylamide sequencing gel containing 8 M urea.
2. Spin the tubes briefly in a microcentrifuge.
3. Heat the tubes 5 minutes at 75°C.
4. Load 1-3 µl (50-200 fmol of Uracil-DNA Excision Mix product) per well.

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