Signosis, Inc.
Innovative Plate Assay Solutions

DNA Damage Extraction Kit

Catalog # EA-7089 **(For Research Use Only)**

Introduction

This kit is used to extract DNA from cell or tissue samples and prepare DNA samples for 8-OHdG DNA damage analysis.

Principle

DNA Extraction

The cell or tissue sample is first lysed to break down the membrane and release the genomic DNA. Then, enzymes are added to the DNA to digest and remove proteins, lipids, and other contaminants from the genomic DNA. The genomic DNA is then washed with ethanol before being resolubilized in TE buffer.

DNA Preparation

The DNA sample is first treated with DNase and nuclease to break down the double-stranded DNA into oligonucleotides and mononucleotides. Then, alkaline phosphatase is added to the digested DNA to remove the 5' end phosphate group of the deoxynucleotide, which prevents self-ligation of the 5' end and 3' end and keeps the deoxynucleotide in a linear form.

Materials Required but Not Provided

- Absolute Ethanol

Materials Provided

- DNA Lysis Buffer (4°C)
- Enzyme Mix I (-20°C)
- TE Buffer (4°C)
- Enzyme Mix II (-20°C)
- Alkaline Phosphatase (-20°C)

****Spin down small tubes before starting experiment. ****

Assay Procedure

DNA Extraction

1. a. For cell samples, collect about $1x10^6$ cells by centrifugation at 2,000 rpm for 5 minutes.
   b. For tissue samples, weigh about 5 mg of tissue and grind the tissue into a fine powder in liquid nitrogen. Transfer the tissue powder to a microcentrifuge tube.
2. Add 40 µL of DNA Lysis buffer to the cell or tissue sample and mix by vortexing.
3. Incubate the sample on ice for 1 minute.
4. Mix the sample by vortexing and then centrifuge the sample in a microcentrifuge at max speed for 3 minutes. The pellet is the isolated nuclei.
5. Remove the supernatant and resuspend the pellet in 40 µL of DNA Lysis buffer.
6. Add 5 µL of Enzyme Mix I to the sample and mix by pipetting up and down.
7. Incubate the sample at 50°C for 1 hour or until the sample solution becomes clear.
8. Add 100 µL of absolute ethanol to the sample and mix by vortexing.
9. Chill the sample at -20°C for 10 minutes.
10. Centrifuge the sample in a microcentrifuge at max speed for 5 minutes.
11. Remove the supernatant from the pellet (isolated DNA) and wash the DNA pellet twice with 70% ethanol.
12. Remove any trace amount of ethanol using a pipette and air dry the DNA pellet for 5 minutes.
13. Resuspend the DNA pellet in 20 µL TBE buffer.
14. Proceed to the DNA preparation step or store the DNA sample at -20°C for future use.

DNA Extraction

1. Add 2 µL of Enzyme Mix II per 50 µg of DNA and incubate at 37°C for 1 hour. This step digests the DNA to form oligonucleotides and mononucleotides.
2. Add 2 µL Alkaline Phosphatase per 50 µg of DNA and incubate at 37°C for 1 hour. This step prevents self-ligation by removing the 5'ends of the deoxynucleotides.
3. The DNA samples can now be assayed using the 8-OHdG DNA Damage ELISA Kit (EA-7085) or stored at -80°C for future use.