

Two-Step Genomic DNA™ Isolation Kits

Website Link: http://fivephoton.com/index.php?route=product/product&path=20&product_id=71

For research uses only. Not a drug.

Caution: Contains an alkaline solution. Avoid eye and skin contact, inhalation and ingestion. Use gloves and eye protection. Wash with copious amounts of water if in eyes or skin. Seek medical attention if ingested.

Prevent exposure to contaminating DNA, by using gloves, autoclaved or UV treated supplies.

Provided Materials:

1. GDIS-1 Solution: 120 mls
2. GDIS-2 Solution: 24 mls
3. Protocol

Required materials not provided:

1. DNA-free 1.5 ml microcentrifuge tube.
2. Microcentrifuge.
3. Heating block.
4. UV Spectrophotometer.

Two-Step Genomic DNA Isolation Kit™: Overview

Kits are suitable for 200 swabs.

- Very simple and rapid method to isolate genomic DNA for SNP analysis.
- By using standard buccal swabs, DNA can be isolated in less than 10 minutes.
- Only two steps are required for DNA yields suitable for PCR amplification.
- Ideal for human (and other mammalian) genotyping and SNP and analysis.
- Excellent substitute for blood draw or cell isolation for genetic analysis.
- The extracted genomic DNA sample from one swab is suitable for 70+ PCR reactions.

Genomic DNA Isolation: Two Step Genomic DNA Isolation™ Kit:

Genetic variability is the hallmark of inheritance and phenotypic diversity. Analysis of genomic DNA sequences allows for detection of genetic variation, and can be applied toward tracing ancestry, paternity and forensics as well as Single Nucleotide Polymorphisms (SNPs) analyses that have been extensively employed to identify markers for human disease prognosis. The FIVEphoton Biochemicals Two-Step Genomic DNA Isolation Kit™ provides a very rapid and simple method to isolate genomic DNA from buccal swabs (mouth swabs) that is applicable for genotyping, paternity, forensic, ancestry STR and SNP analysis. By using the FIVEphoton Biochemicals Two-Step Genomic DNA Isolation Kit, genomic DNA for PCR amplification can be isolated in less than 10 minutes. This is a substantially shorter time compared to widely marketed spin column techniques. The FIVEphoton Biochemicals genomic DNA isolation method can also be completed in 96-well dishes, which enables large scale genomic analysis.

Principles of the Kit

In the buccal swab method for genomic DNA collection, individuals simply swipe their inner mouth several times using a swab made of various materials and configurations. The swab can be stored at room temperature prior to genomic DNA extraction. PCR primers are designed that bracket the region or nucleotide position of interest to analyze segments or specific nucleotide positions. PCR is then employed to amplify the DNA. DNA sequencing or sizing chromatography methods are employed to detect individual genetic variations.

Genomic DNA Isolation using the Two-Step Genomic DNA Isolation™ Kit. A Synopsis

After swabbing the inner mouth, the individual carefully places the swab in a sterile packaging that prevents further exposure to DNA. The swab can be sent in the mail to the processing laboratory if genomic DNA isolation procedures are done remotely. The

genomic DNA is stable during an extended mailing period. Once the swab reaches the processing laboratory, the swab is cut or removed from its handle and placed in Genomic DNA Isolation Solution 1 (GDIS-1) in a 1.5 ml tube and heated at 95 degrees C for 5 minutes. The solution is cooled on ice and then genomic DNA Isolation Solution 2 (GDIS-2) is added to the tube, which is vortexed. The swab is removed using a forcep that has been flamed (or sterilized) to remove contaminating DNA. The extraction method yields a 720 μ l volume of genomic DNA solution: only 5-10 μ l of this solution is required for each PCR amplification.

The isolated genomic DNA can be stored at -20 degrees C for an extended time period prior to PCR amplification. The entire genomic DNA isolation process requires less than 10 minutes and is performed in two steps, which represents a substantially more time efficient method than commercially available kits that employ spin column isolation.

The absence of blood collection and the ease of genomic DNA isolation provided by the FIVEphoton Biochemicals Two-Step Genomic Isolation KitTM facilitates wide scale genetic analysis to academic and commercial researchers.

Protocol for Genomic DNA Isolation

Use gloves and eye protection in all procedures. Prevent contamination from exogenous DNA.

1. Dispense 600 μ l of GDIS-1 solution into a 1.5 μ l DNA-free microcentrifuge tube.
2. Cleave off or eject the swab from the handle into the DNA-free 1.5 ml microcentrifuge tube with 600 μ l of GDIS-1. Vortex the tube for 10 sec, and then heat to 95°C for 10 minutes (make sure the tub cap is secure and can not open during heating). Remove the swab.
3. Add 120 μ l of GDIS-2 and vortex again for 10 sec. This final solution contains the

extracted genomic DNA that can be used as a template for PCR amplification. Store the genomic DNA solution at -20 degrees C. Thaw before use.

4. Use a spectrophotometer to measure the 260/280nm absorption ratio to determine purity and concentration. The appropriate 260/280 nm ratio for subsequent PCR amplification should be approximately 1.8-2.5. You can also analyze the extracted genomic DNA using agarose gel electrophoresis and cleavage with a restriction enzyme such as *psf-1*.

Resources for genomic DNA sequences and PCR primer design.

Genomic DNA Database: UCSC Genome Browser: <http://genome.ucsc.edu/>

PCR Primer Design: Primer3: <http://frodo.wi.mit.edu/primer3/>

Sequence Analysis: NCBI Blast: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>