Extracting circulating DNA from serum/plasma samples

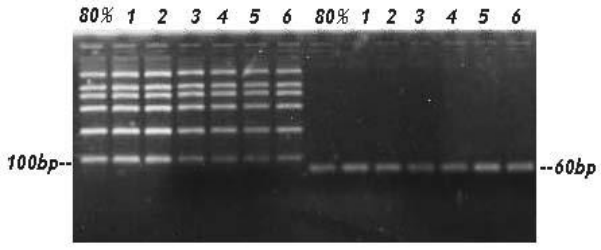
Introduction

Circulating DNA is extracellular DNA that exists in body fluids such as blood (plasma or serum) and cerebrospinal fluid. It is mainly composed of single or double-stranded DNA and mixture of single and double-stranded DNA, and exists in two forms: DNA protein complexes or free DNA. As early as 1947, Mandel and Metais discovered circulating nucleic acids. Thirty years later, Leon's study showed that the peripheral serum DNA levels of tumor patients were significantly higher than those of normal individuals; In 1989, Stroun discovered that blood free DNA had some characteristics of tumor cell DNA. Five years later, researchers detected oncogene mutations in the plasma and serum of tumor patients, which were consistent with the primary tumor. With the progress of tumor molecular biology research, the detection of circulating blood free DNA and the study of its biological indicators will provide a series of convenient, fast, specific, non-invasive or minimally invasive molecular biology detection methods for early diagnosis, prognosis judgment, and follow-up of clinical tumors. In lung cancer and breast cancer, point mutation of circulating DNA in serum and drug regimen has been applied clinically. Magen's HiPure Circulating DNA Kits are specifically designed for the extraction of cell-free DNA from serum and plasma. The kit uses silica gel column purification technology and can process 0.1-5ml of samples. The purified DNA can be directly used for fluorescence quantification, SNP detection, liquid chip analysis, gene chip analysis, etc.

Experiment Results

1. DNA recovery of different fragments

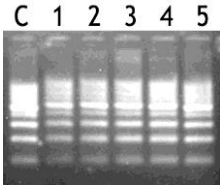
Take 20μl 100bp DNA Marker and 20μl 60bp PCR products, diluted with serum to 500μl, extract using HiPure Circulating DNA Micro Kit, and finally wash out the DNA using 20μl Elution Buffer. After 2% agarose gel electrophoresis, analyze the recovery rate of original product (80%) and the recovered product. As shown in the figure, Magen kit can efficiently recover DNA fragments of various sizes, with a recovery rate over 80%.



2. DNA recovery of large volume sample

Take 20μl 100bp DNA Marker, diluted with plasma to 1ml, 2ml, 3ml, 4ml, and 5ml, extract according to HiPure Circulating DNA Midi Kit, and finally wash out the DNA with 20μl Elution Buffer. After 2% agarose gel electrophoresis, analyze the recovery rate of original product (80%) and the recovered product.

As shown in the figure, when the DNA concentration continues to decrease, the recovery efficiency of different fragments can still reach 80%.

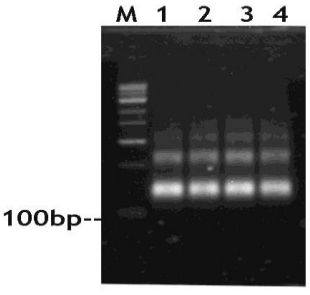


3. Extraction of free DNA from chicken blood

Take 3 ml of chicken blood serum, extract according to HiPure Circulating DNA Midi Kit, and finally wash out free DNA with 50μl Elution Buffer. The obtained DNA was measured by Nanodrop 2000, and the results are as follows. It can be seen that a large amount of free nucleic acids, approximately 9-13μg, can be obtained from 3ml chicken serum. The purified DNA has high purity, with an OD260/OD280 of approximately 1.8-1.9.

Sample	Conc. μg/μl	A260	A280	260/280	260/230	Yield μg
1	277.9	5.558	2.879	1.93	2.09	13.90
2	280.7	5.613	2.903	1.93	2.12	14.04
3	204.4	4.088	2.193	1.86	1.46	10.22
4	197	3.94	1.811	1.87	2.18	9.85

Take 5μl purified chicken serum DNA for electrophoresis analysis on 2% agarose gel. Results are as follows. As shown in the figure, the fragment size of free DNA in chicken serum is between 150bp and 1000bp.



Note: Chicken serum contains abundant free nucleic acids, mainly because red blood cells in chicken blood have nucleus. Research has shown that the content of free DNA in human serum is very low, with 1ml of human serum containing approximately 1ng-100ng of free DNA.