

Nucleic acid preparation

DNA preparation

Technical tip

DNA/RNA purification

Extraction and purification of nucleic acids involve different methods :

- Extraction methods from complex biological samples starts generally with a step of **disruption** or **lysis** of cell and organelles membranes (to release of DNA from nucleus mitochondria, and RNA from cytoplasm), followed by a **separation** step discarding cellular debris (usually performed by centrifugation). Membranes can be broken with french press, and cell lysis can be accomplished detergents or chaotropic agents, with inactivation of the cellular nucleases.

- A standard extraction and purification method involve **partition of nucleic materials between different liquid phases** (and eventually proteins and lipids). Traditionally, phenol or phenol:chloroform are used to separate the nucleic acids, into the aqueous phase, from the other cellular components including proteins, found in the organic phase. New techniques are now available which avoid the use of phenol (a toxic product).

- A second method consists in **precipitation of DNA/RNA by chemicals**, especially organic solvents as ethanol or isopropanol. This allow to concentrate nucleic acids in the pellet, and to discard impurities from supernatant. DNA can be washed and easily resolubilized. Enzymatic treatment may be useful to degrade contaminating proteins (with proteases), or undesired RNA (with RNases).

- An alternative convenient method relies on **solid phase extraction of nucleic acids** on a matrix, i.e. silica. Adsorption occurs through hydrophobic and ionic interactions. Extensive washes can be performed to remove other components, before elution in a suitable buffer.

- Specific applications use **affinity based methods**, i.e. avidin grafted supports for biotinylated primers.

Many methods are available to extract and purify nucleic acids, depending on :

- ◆ starting material; this include complex biological samples (tissues, cells, bacteria, virus...) and *in vitro* preparations (amplifications reactions, affinity chromatography fractions...)
- ◆ desired nucleic material: DNA, cDNA, plasmids, RNA, mRNA,...
- ◆ the goal: to isolate, concentrate or desalt nucleic material. The purity and quality of DNA/RNA, usually estimated with an OD260/280 measurement, should suit downstream applications, including analysis, PCR amplifications, diagnostics or therapeutics.

Interbiotech offers basic chemical reagents for extraction of nucleic acids, as well as kits, based mainly on silica matrix or ionic exchange, that make easier process of specific applications including difficult samples or demanding genetic techniques as DNA amplification, RT-PCR, or transfections.

See also :

Desalting/dialysis, electroelution

DNA/RNA labelling

DNA Preparation - Cells & Tissue

BDtract™ Genomic DNA Isolation Kit

Purify DNA, free of RNA, proteins, and degrading enzymes

Sample Source : Whole blood, Cultured cells, Tissue, Bacteria, PCR Product

Sample Size :

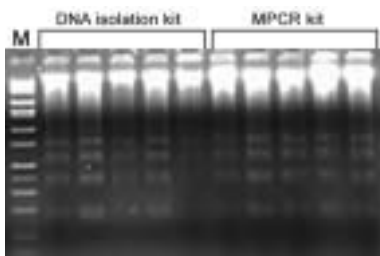
- Cells grown in suspension : 5-10 x 10⁶ cells
- Cells grown in monolayer : 100 mm culture dish
- Tissue : 50-100 mg
- Bacteria : 1-5 ml
- Blood : 2.5 ml

Preparation Time : Approximately one hour

- ◆ Economical, Fast, and Simple
- ◆ High DNA Yield : 30-40 µg/mL blood
- ◆ Ultra Purity : A₂₆₀/A₂₈₀ ratio of 1.8-1.9
- ◆ Low contamination : No RNA, Protein, or degrading enzyme contamination
- ◆ Non-toxic : No phenol, chloroform, or other toxic extractions
- ◆ Stable : Reagents stored at room temperature

Genomic DNA Isolation Kit provides all the necessary reagents and protocols for quickly extracting high-molecular-weight DNA from whole blood, cultured cells, tissue, and bacteria. This kit precludes the need for phenol, chloroform or other organic extraction. RNA is removed by treatment with DNase-free RNases. Proteins are further removed by salt precipitation. Genomic DNA isolation is achieved through alcohol precipitation and then dissolved in TE buffer. The purified DNA is free of RNA, proteins, and degrading enzymes, and may be used directly for RFLP, restriction digests, cloning, Southern blotting, PCR amplification, and other DNA analysis techniques.

Description	Cat.#	Qty
BDtract™ Genomic DNA Isolation Kit	T66251	50 tests
BDtract™ Genomic DNA Isolation Kit	T66250	100 tests



Blood Samples were stored at 4°C for 2 months. Genomic DNA was purified using Genomic DNA Isolation Kit (Left Figure).

100 ng of each DNA was performed with DMD/BMD MPCR Kit # T56180 (Right Figure).

M : 100 bp Ladder DNA M.W. Marker
lane 1-10: individual patient #1-10.γ

Kit Components : Cell lysis buffer, RBC wash buffer, Nuclei lysis buffer, RNase solution, Protein, precipitate solution, Optimized protocol

TissueDirect Multiplex PCR System

Sample Source : Animal (such as mouse) tails, tissues (fresh, frozen, or paraffin), microdissection, buccal cells, hair shaft, saliva, sperm, and cells.

- Sample Size : Tissue : 5 mg
 - Saliva : 15 µl
 - Cells culture : 15 µl
- ◆ Easy to perform : very simple and rapid procedure to extract genomic DNA in 12 min.
 - ◆ High specificity : highly specific amplification of genomic DNA using "HotStart" Script™ DNA polymerase
 - ◆ Multiplex PCR : up to >1 000 DNA sequences can be amplified using multiplex PCR primers.
 - ◆ Super sensitivity : genomic DNA from a single sperm has been successfully used in multiplex PCR amplification of more than 1000 amplicons and subsequent DNA genotyping assays. The super sensitivity of this kit will dramatically cut down the tissue utility to save your precious tissue samples. This kit will also allow you to use less invasive method for genomic DNA preparation needed for genotyping.

TissueDirect™ Multiplex PCR System is a powerful reagent kit for both easy and rapid genomic DNA preparation and multiplex PCR amplification. Genomic DNA is directly released from cells (tissues, mouse tails, hair shafts, cell culture) using proprietary reagents in 12 minutes without DNA isolation. The genomic DNA can be used immediately in PCR amplification of multiple gene targets (up to >1,000) or stored at + 4 °C for future use.

For applications such as :

- ◆ SNP genotyping and mutation detection
- ◆ Target detection in transgenic mice
- ◆ DNA sequencing and cloning
- ◆ Quantitative PCR

Description	Cat.#	Qty
TissueDirect Multiplex PCR System (without Enzyme)	BM6190	100 Preps
TD-A Buffer, TD-B Buffer, TD-C, TD-D Buffer, PCR-grade water		
TissueDirect Multiplex PCR System (with Enzyme)	BM6200	100 Preps
TD-A Buffer, TD-B Buffer, TD-C, 2X PCR Premix, PCR-grade water		

DNA Preparation - Blood

Blood DNA Sample Preparation Column Kit

DNA directly from blood to PCR

Sample Source : Fresh or frozen whole blood

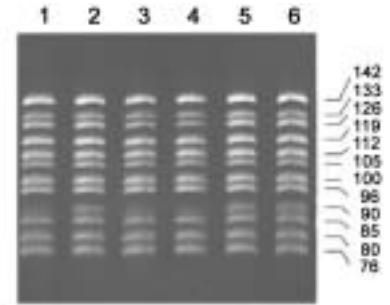
Sample Size : 500 µl

Elution Volume : 2 x 100 µl

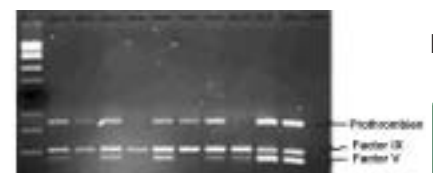
The Blood DNA Sample Preparation Column Kit offers a quick and easy way to isolate DNA from whole blood samples using isolation methods based on binding nucleic acid to column membrane. The purified DNA can be used directly for PCR amplification. The following example is demonstrated by PCR amplification with allelic specific primers and column isolated blood DNA samples.

Description	Cat.#	Qty
Blood DNA Sample Preparation Column Kit	T66311	50 tests
Blood DNA Sample Preparation Column Kit	T66310	100 tests

Kit Components : Spin Columns, BD-1 Solution, Lysis Solution, Wash Buffer, Elution Solution



Multiplex PCR Amplification of Human Genomic DNA Extracted Using TissueDirect™ Multiplex PCR System. PCR DNA sizes are shown on the right. Lane 1 and 2 using 30 HEK293 cells. Lane 3 and 4 using breast cancer microdissection. Lane 5 and 6 using human hair shafts.



Genomic DNA quality control validation of human thrombosis MPCR Kit
M : 100 bp Ladder DNA M.W. Marker
lane 1 : PCR using wild type MPCR primers with patient #1 DNA

Nucleic acid preparation

DNA preparation

IsoQuick™ Nucleic acid extraction kit

The shortest way from blood to PCR

Sample Source : recommande for whole blood, but can work on other samples

Sample Size : 100 µl

The IsoQuick™ nucleic acid extraction kit is intended for use in the extraction and purification of nucleic acid from a variety of complex biological materials, such as whole blood, bacteria or culture cells. This kit uses a simple and highly effective phase separation technique. Based on guanidine thiocyanate, it provides the researcher with DNA and/or RNA of purify and yield comparable with phenol/chloroform method, but without the use of hazardous chemicals. The three step process gives the researcher PCR or enzyme digestion ready DNA in 20 mn (or RNA in 40 mn). This kit requires no special storage.

Kit Components : Lysis Solution, Extraction Matrix, 20X Dye Concentrate, Extraction Buffer, Sodium Acetate, RNase-free Water, Sample Buffer

Description	Cat.#	Qty
IsoQuick™ Nucleic acid extraction kit	172080	100 tests

MonoFas mini DNA Blood Kit

Extraction of genome DNA from 2 µl of whole blood

Sample : For 2 – 10 µl of whole blood

Applications : PCR amplification, restriction enzyme digestion, virus, bacteria/fungal analysis, cancer research, human genetic tests, forensic medicine tests.

- ◆ Fast extraction and purification in 10 minutes
- ◆ Clean and high recovery : A260/280 > 1.7
- ◆ Elution buffer is sodium free
- ◆ Elution by only 2 µl is possible
- ◆ High purity eluent can be applied directly for efficient PCR amplification.

This kit is designed for the purpose of DNA purification from valuable or trace amounts of blood, biological fluids and blood stains.

Adaptive analytes are such as fresh and frozen whole blood (anticoagulant), buffy coat, bone marrow fluid, lymphocytes, leucoctyes, biological fluids.

Monolith Silica

specification :

Through pore diameter : 15 µm

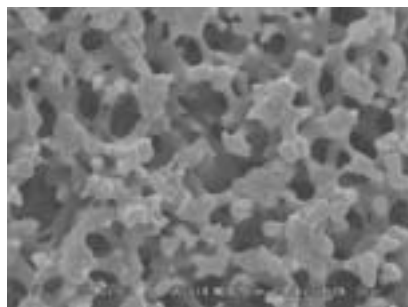
Meso-pore : 10 nm

Fluorescent sequencing can be used :

Sample : DNA to make it amplify from human genome by using Takara PCR Kit .

Sequence data analyzed with ABI Prism 3730 Genetic Analyzer

Description	Cat.#	Qty
MonoFas mini DNA Blood Kit	BN3310	50 tests
	BN3311	100 tests



Simple!!! Only 3 steps

Blood (2 – 10µL)



Add 2 volumes of Binding Buffer A and dissolve at 70C, 5min



Bind



Centrifuge for a minute at 10,000rpm



Wash

Washing Buffer B
50µL



Centrifuge for a minute at 10,000rpm



Elute

Elution Buffer C
2 – 5µL



Centrifuge for a minute at 10,000rpm



Elute

Purified genome DNA

DNA extractor kit

Detects and quantitates contaminant DNA in serum and residual DNA in biopharmaceuticals

- ◆ Avoid toxic organic solvents
- ◆ High quality and high recovery from biologicals fluids

Sample Source : human serum

Sample Size : 100 µl (50 reactions), 200 µl (25-30 reactions)

In the detection and measurement of DNA in fluids, DNA must be isolated from the proteins in the sample.

The "DNA extractor kit" employs a new extraction procedure for DNA purification from human serum in a single tube. This procedure using Sodium Iodide (NaI) as a chaotropic agent realizes DNA isolation of both high quality and high recovery from biologicals fluids without complex and laborious manipulations.

A high concentration of chaotropic reagent, NaI, and an anionic detergent participate in solubilization of the proteins and lipids contained in biological samples. After addition of isopropanol to the mixture, nucleic acids are co-precipitated with polysaccharide glycogen as a carrier, while other components remain soluble in the solution phase.

Reference : Ishizawa M. et al.: "Simple procedure of DNA Isolation from Human Serum", *Nucleic Acids Res.*, 19, 5792 (1991).

Description	Cat.#	Qty
DNA extractor kit	095330	50 tests

DNA Preparation - Plasmid

Plasmid DNA Isolation Column Kit

From Bacteria to the highest purified DNA for many applications

Sample Source : Bacterial cell culture

Sample Volume : 1.5 - 5 ml

Elution Volume : 60 x 100 µl

The Plasmid DNA Isolation Column Kit offers a quick, efficient, and convenient means to extract high quality DNA from bacterial cell culture through purification methods based on binding nucleic acid to column membrane. Extracted DNA can be used directly for a variety of genetic applications without further manipulation.

Description	Cat.#	Qty
Plasmid DNA Isolation Column Kit	T66351	50 tests
Plasmid DNA Isolation Column Kit	T66350	100 tests

CCL (Complete Cell Lysis Solution)

CCL is a ready-to-use solution for lysis of bacterial cells and removal of RNA from plasmid DNA mini-preps. CCL, stable at 4°C, can be substituted directly for lysozyme stock solutions in most alkaline lysis and boiling mini-preps. Because there is no weighing out of small fractions of lyophilized powders, no preparation of stock solutions, and no thawing of reagents before use, CCL provides maximum convenience.

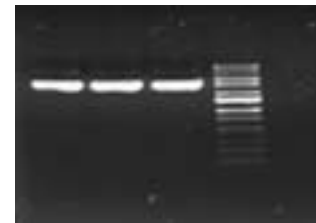
RNase A : 10 mg/ml

Lysozyme : 10 mg/ml

DNase : None Detected

Stability (4°C) : > 6 months

Description	Cat.#	Qty
Complete Cell Lysis Solution	741470	5 ml



Three genotypes of pASA plasmid DNA were isolated using *Plasmid DNA Isolation Kit*.

Lane 1 : Wild-Type factor V gene (Genotype G/G)
Lane 2 : Hetero-Type factor V gene (Genotype G/A)
Lane 3 : Homo-Type factor V gene (Genotype A/A)

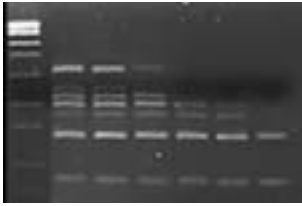
Kit Components : Spin Columns, Resuspension Solution, Lysis Solution, Neutralization Solution, Wash Buffer, Elution Solution

Related product :

Description	Cat.#	Qty
Terrific Broth	821111	500 g

Nucleic acid preparation

PCR and labeled probes clean-up



MPCR amplicons are purified by PCR Cleanup kits
 Lane M : 100 bp Ladder DNA M.W. Marker
 Lane 1 : Original MPCR amplicons with different sizes
 Lane 2 : PCR Cleanup Column Kit
 Lane 3 : Supplier A
 Lane 4 : Supplier B
 Lane 5 : Supplier C
 Lane 6 : Supplier D

Kit Components : Spin Columns, Resuspension Solution, Binding Buffer, Wash Buffer, Elution Solution

Related products : DNA labeling

- ◆ Label IT® Nucleic Acid Labeling Kits, available with digoxin, biotin, DNP (dinitrophenyl) labels, and with fluorescein, CR-rhodamine, TR-rhodamine, and Cy™3 or Cy™5 dyes.
- ◆ Label IT® Nucleic Acid Modifying Kits (amino and carboxyl).

PCR Cleanup Column Kit

Get pure DNA after amplification

Sample Source : PCR Product

Sample Size : 50 µl

Elution Volume : 30 x 50 µl

The PCR Cleanup Column Kit offers a quick, efficient, and convenient means to extract high quality DNA from PCR amplified products through purification methods based on binding nucleic acid to column membrane. Extracted DNA can be used directly for a variety of genetic applications without further manipulation.

Description	Cat.#	Qty
PCR Cleanup Column Kit	T66371	50 tests
	T66370	100 tests

PrestoSpin D – DNA Purification Mini Spin Columns

- ◆ New Technology
- ◆ Superior DNA quality
- ◆ High yield
- ◆ Rapid protocols

This total nucleic acid and DNA purification kit relies on a new nucleic acid binding mechanism. According to this, negatively charged DNA and RNA molecules interact with negatively charged surfaces by a complexing reaction involving multivalent cations such as magnesium. DNA is released as the result of removal of magnesium by EDTA. This reversible binding mechanism is made use for purification of nucleic acids from crude cell extracts. As a binding matrix clay minerals are used, which have an extremely high binding capacity. The new product, named PrestoSpin D, is a kit series consisting of mini spin columns filled with a mixture of pure sand and clay and all reagents needed for cellular lysis and nucleic acid purification. With PrestoSpin D nucleic acids can be extracted and purified from bacteriophages, bacteria, fungi and yeasts, plants, soil, tissues, food/feed, blood and cell cultures. An absolutely new feature of PrestoSpin D is the purification of plasmid DNA in midi format, cosmid and BAC DNA, using mini spin column purification. The PrestoSpin D nucleic acid purification procedures are quick, have low hands-on times and are an economic alternative.

PrestoSpin D Universal – all-in-one kit : genomic and plasmid DNA.

PrestoSpin D Lambda – Lambda and other phages : yield up to 15 µg (10 ml culture), time 1 h 10 min.

PrestoSpin D Bug – Gram-negative and Gram-positive bacteria : yield up to 80 µg (2.10¹⁰ cells = 5 ml, time 35 min).

PrestoSpin D Fungi – yeasts and fungi : 7 µg (1 ml yeast culture), time 40 min to 1 h 10 min.

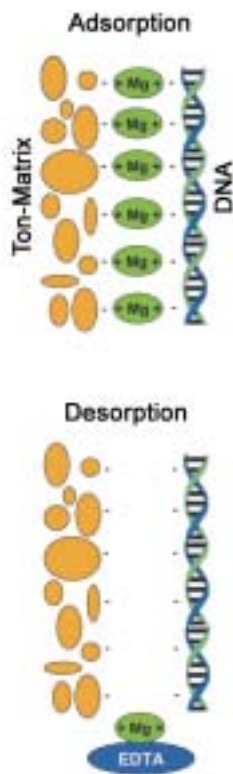
PrestoSpin D Plant – plants and soils : 12,5 µg (100 mg wheat), time 50 min.

PrestoSpin D Food – animal and plant origin food/feed : 1,9 µg (20 mg flour) to 10 µg (500 mg liver sausage), time: 50 to 90 min.

PrestoSpin D Tissue – animal and human tissue, mouse tail : up to 60 µg (brain), time 90 -120 min (complete digestion).

PrestoSpin D Blood&Cell – Blood and cell culture : 2.7 µg (100 µl blood) – 19.5 µg (10⁷ cultured cells).

PrestoSpin D Plasmid – Plasmid Midi Format : up to 50 µg (high copy), time 45 min. OD260/280 nm : 1,7 – 2,0 ; Mr genomic DNA : > 50 kb ; Plasmid DNA: >99%.

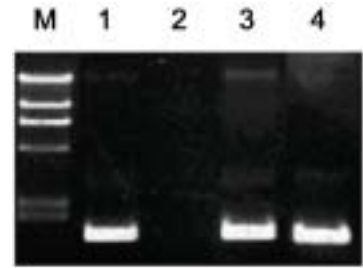


DNA binding by cation complexation. Impurities are washed out and DNA is eluted, without ethanol precipitation, using TE buffer.

Nucleic acid preparation

PCR and labeled probes clean-up

Description	Cat.#	Qty
PrestoSpin D Universal Genomic and plasmid DNA All-in-one kit	U20450	50 Tests
	U20451	250 Tests
PrestoSpin D Plasmid high and low copy plasmids - cosmids, BACs	AA2940	25 Tests
	AA2941	100 Tests
PrestoSpin D Plant Plants and soil	AM3250	50 Tests
	AM3251	250 Tests
PrestoSpin D TissuE Tissue incl. mouse tail	AM3260	50 Tests
	AM3261	250 Tests
PrestoSpin D Lambda Lambda and other bacteriophages	AM5560	25 Tests
	AM5561	100 Tests
PrestoSpin D Bug Gram-positive/Gram-negative bacteria	AM5570	50 Tests
	AM5571	250 Tests
PrestoSpin D Fungi Yeasts and fungi	AM5590	50 Tests
	AM5591	250 Tests
PrestoSpin D Food Food/feed	AM5600	50 Tests
	AM5601	250 Tests



Midi isolation of plasmid DNA (pUC13) using PrestoSpin D Plasmid kit. Left: 1, eluate; 2, flow-through after column loading; 3, total plasmid DNA; 4, plasmid-DNA isolated with a kit of a competitor (anion exchange).

Right: Plasmid screening of wild bacterial strains using PrestoSpin D Plasmid. Plasmids of >50 kb (arrow) can be isolated.

DNA/RNA recovery from electrophoresis gels (electroelution)

Please see 'GebaFlex' products in chapter B = Proteomics, that are efficient tools to recover nucleic acids from electrophoresis gels. Great applications are :

◆ recovery of large DNA fragments >10kb

HAND OFF WITH TEDIOUS DNAs

For >10kb DNAs, the bead technology purifications dont work properly. GebaFlex method offers a superior method to conventional elution that operates by diffusion overnight from agarose pieces. GebaFlex procedure takes only 15minutes, and nucleic acids can be directly desalted, or purified by beads technology, for any downstream applications. To get i.e. 4 OD of DNA, you need much less (up 2 fold less) starting DNA because yield ranges 80 to 90% (see figure).

Large DNA fragment Electro Elution method with GeBAflex-tube

◆ recovery of small DNA (oligos)

DON'T GO TO HPLC !

GebaFlex offers an excellent alternative method to HPLC purification of oligonucleotides (cheap, and too time consuming and low yield diffusion elutions. Scale up is easier then with HPLC

Compared with :

HPLC purification of oligonucleotides diffusion elutions

GebaFlex method has following advantages :

cheaper, no development, scale up is more easy much higher yield, quicker

GebaFlex offers an excellent alternative method to HPLC purification of oligonucleotides (cheaper, scalable), and to time consuming and low yield diffusion elutions.



Large DNA fragment Electro Elution Method with GeBAflex-tube

